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# Ecotoxicological Test Systems

## Proceedings of a Series of Workshops

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# ECOTOXICOLOGICAL TEST SYSTEMS

## PROCEEDINGS OF A SERIES OF WORKSHOPS

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DEPARTMENT OF ENERGY

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# FOREWORD

The scientific disciplines of ecology and environmental toxicology have not been communicating adequately with each other, to the detriment of both. Ecologists are often falling short when it comes to applying the theory and findings of their relatively young science in useful practice to meet society's needs for assessment of the environmental impacts of toxic pollutants. Environmental toxicologists are increasingly having difficulty in trying to convince society's decision makers what the results of their test methodologies in simple systems really mean in a complex, highly interactive ecological world.

These workshops take a step toward marrying some of the concepts of these two scientific disciplines. At the request of the Environmental Protection Agency's Office of Toxic Substances, the Environmental Sciences Division of Oak Ridge National Laboratory has convened this series of workshops to review and evaluate potential techniques for studying ecological effects of toxic chemicals in systems that transcend the practicable but oversimplified conditions of most currently used toxicological test systems.

EPA intends to use this study, and companion efforts, to help guide our future attempts to bring about better synergy between ecology and environmental toxicology in our implementation of the Toxic Substances Control Act.

James J. Reisa

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# ABSTRACT

A series of six workshops was conducted by the Environmental Sciences Division, Oak Ridge National Laboratory, to identify laboratory methods and data evaluation techniques for predicting the environmental effects of chemical substances. Methods were evaluated for their potential for standardization and for use in the ecological hazard and risk assessment processes under the Toxic Substances Control Act. The workshops addressed assessment and policy requirements of multispecies toxicology test procedures, mathematical models useful in hazard and risk assessments, and methods for measuring effects of chemicals on terrestrial and aquatic population interactions and ecosystem properties. The workshops were primarily used as a mechanism to gather information about research in progress. This information was part of the data base used to prepare a critical review of laboratory methods for ecological toxicology.

This report was submitted in partial fulfillment of Interagency Agreement No. EPA 78-D-X0387 between the Department of Energy and the U.S. Environmental Protection Agency.

# CONTENTS

Page

FOREWORD . . . iii

ABSTRACT . . . v

TABLES . . . xi

FIGURES . . . xii

ACKNOWLEDGMENTS . . . xiii

1. INTRODUCTION . . . 1

2. CONCLUSIONS AND RECOMMENDATIONS . . . 5

    2.1 Introduction . . . 5
    2.2 Terrestrial Test Systems . . . 6
        2.2.1 Population Interactions . . . 6
        2.2.2 Ecosystem Properties . . . 6
    2.3 Aquatic Test Systems . . . 7
        2.3.1 Population Interactions . . . 7
        2.3.2 Model Ecosystems . . . 8
    2.4 Mathematical Models . . . 9

3. ASSESSMENT AND POLICY REQUIREMENTS OF MULTISPECIES TOXICOLOGY TESTING PROCEDURES . . . 11

    3.1 Introduction . . . 13
    3.2 Results and Discussion . . . 13
    3.3 Conclusions . . . 15

4. MATHEMATICAL MODELS USEFUL IN TOXICITY ASSESSMENT . . . 19

    4.1 Introduction . . . 21
    4.2 Results and Discussion . . . 22
        4.2.1 Terrestrial Simulation Models . . . 25
            (1) Regional Biogeochemical Models . . . 29
            (2) Radiological Cycling and Transport Models . . . 29
            (3) Global Biogeochemical Models . . . 29
            (4) Forest Succession Models . . . 30
            (5) Theoretical Ecosystem Model . . . 31
        4.2.2 Aquatic Simulation Models . . . 31
            (1) Ecosystem Models . . . 31
            (2) Fate Models . . . 33
            (3) Fate-and-Effects Models . . . 33

## CONTENTS (continued)

Page

4.2.3 Generalized Multipopulation Models . . . . . . . . 34
4.2.4 Alternative Methodologies . . . . . . . . . . . . 34
(1) Loop Analysis . . . . . . . . . . . . . . . . . . 34
(2) Time Averaging . . . . . . . . . . . . . . . . . 36
(3) Input-Output Analysis . . . . . . . . . . . . . 36
(4) Natural Selection Models . . . . . . . . . . 37
4.3 Conclusions . . . . . . . . . . . . . . . . . . . . . . . 37
4.3.1 Criteria for Evaluating and Selecting Models . . . . . . . . . . . . . . . . . . . . . 37
4.3.2 Research Priorities . . . . . . . . . . . . . . . 40
4.4 References . . . . . . . . . . . . . . . . . . . . . . . 42

5. METHODS FOR MEASURING EFFECTS OF CHEMICALS ON TERRESTRIAL ECOSYSTEM PROPERTIES . . . . . . . . . . . . . . 49

5.1 Introduction . . . . . . . . . . . . . . . . . . . . . . 51
5.2 Results and Discussion . . . . . . . . . . . . . . . 52
5.2.1 Microbial Processes . . . . . . . . . . . . . . . 52
(1) Identification and Evaluation of Test Systems . . . . . . . . . . . . . . . . 53
(a) Cellulose Decomposition . . . . . . . . 53
(b) Nitrogen Transformation . . . . . . . . 54
(c) Sulfur Transformation . . . . . . . . . 54
(d) Other Existing Test Systems . . . . . 55
(e) Proposed Alternate Tests . . . . . . . . 59
(2) Protocol Development . . . . . . . . . . . . 63
5.2.2 Identification of Model Ecosystems . . . . . . . 65
(1) Test Description . . . . . . . . . . . . . . . 65
(a) Synthetic Systems . . . . . . . . . . . 65
(b) Excised Systems . . . . . . . . . . . . 65
(2) Measurable Parameters . . . . . . . . . . . 66
5.2.3 Evaluation Model Ecosystems . . . . . . . . . . 68
(1) Synthetic Systems . . . . . . . . . . . . . . 68
(2) Excised Systems . . . . . . . . . . . . . . . 69
(3) Uncertainties Concerning All Model Ecosystems . . . . . . . . . . . . . . . 69
5.2.4 Protocol Development for Model Ecosystems . . . 70
5.3 Conclusions . . . . . . . . . . . . . . . . . . . . . . . 71
5.3.1 Microbial Processes . . . . . . . . . . . . . . . 71
5.3.2 Model Ecosystems . . . . . . . . . . . . . . . . 72
5.3.3 General Discussion . . . . . . . . . . . . . . . 72
5.4 References . . . . . . . . . . . . . . . . . . . . . . . 74

6. METHODS FOR MEASURING EFFECTS OF CHEMICALS ON AQUATIC ECOSYSTEM PROPERTIES . . . . . . . . . . . . . . . . . . . . . . 79

6.1 Introduction . . . . . . . . . . . . . . . . . . . . . . 81

CONTENTS (continued)

Page

6.2 Results and Discussion . . . . . . . . . . . . . . . . . . 83
6.2.1 Test Descriptions and Measurable Ecosystem-Level Properties . . . . . . . . . . . . 83
(1) Lentic Working Group . . . . . . . . . . . . 83
(2) Lotic Working Group . . . . . . . . . . . . 87
6.2.2 Evaluation of Test Systems . . . . . . . . . . . . 87
(1) Lentic Working Group . . . . . . . . . . . . 87
(2) Lotic Working Group . . . . . . . . . . . . 93
6.2.3 Protocol Development . . . . . . . . . . . . . . 96
(1) Sediment Core Effects Test Procedure . . . 96
(2) Pelagic Ecosystem Effects Test Procedure . . . . . . . . . . . . . . . . . 98
(3) Model Stream Effects Test Procedure . . . . 99
6.3 Conclusions . . . . . . . . . . . . . . . . . . . . . . . 100
6.4 References . . . . . . . . . . . . . . . . . . . . . . . 101

7. METHODS FOR MEASURING EFFECTS OF CHEMICALS ON TERRESTRIAL POPULATION INTERACTIONS . . . . . . . . . . . . . 105

7.1 Introduction . . . . . . . . . . . . . . . . . . . . . . 107
7.2 Results and Discussion - Microbial Populations . . . . 109
7.2.1 Population Interactions . . . . . . . . . . . . 109
(1) Predation and Parasitism . . . . . . . . . . 109
(2) Competition . . . . . . . . . . . . . . . . 111
(3) Mutualism . . . . . . . . . . . . . . . . . 111
(a) Lichens . . . . . . . . . . . . . . . 111
(b) Methanogenesis . . . . . . . . . . . . 111
(4) Antagonism . . . . . . . . . . . . . . . . 112
(a) Test Procedure . . . . . . . . . . . . 113
(b) Analysis of Results . . . . . . . . . . 113
(c) Development Needs . . . . . . . . . . 115
7.2.2 Community Properties . . . . . . . . . . . . . . 115
(1) Survival Test . . . . . . . . . . . . . . . 115
(2) Fungistasis . . . . . . . . . . . . . . . . 117
(3) Population Levels . . . . . . . . . . . . . 118
(4) Succession . . . . . . . . . . . . . . . . 119
(5) Diversity . . . . . . . . . . . . . . . . . 119
7.3 Results and Discussions - Plant and Microbe Populations . . . . . . . . . . . . . . . . . . . . . 120
7.3.1 Interference . . . . . . . . . . . . . . . . . . 120
7.3.2 Mycorrhizae - Plant Interactions . . . . . . . . 123
(1) Endomycorrhizae - Grass . . . . . . . . . . 123
(2) Ectomycorrhizae - Conifer . . . . . . . . . 124
7.3.3 Rhizobium - Legume Interaction . . . . . . . . . 124
7.3.4 Wheat - Wheat Rust . . . . . . . . . . . . . . . 125
7.3.5 Carrot - Crown Gall . . . . . . . . . . . . . . . 125
7.3.6 Plant - Nematode Interaction . . . . . . . . . . 125
7.3.7 Agricultural Soil Microcosm . . . . . . . . . . . 125

CONTENTS (continued)

Page

7.4 Results and Discussion - Arthropod Interactions . . . . 126
  7.4.1 Proposed Test Systems . . . . . . . . . . . . . 126
    (1) Plant - Whitefly - Parasitoid . . . . . . . 129
    (2) Corn - Earworm - Exploiters . . . . . . . . 129
    (3) Alfalfa - Aphid - Parasitoid . . . . . . . 129
    (4) Plant - Brown Scale - Exploiters . . . . . 129
    (5) Housefly - Blowfly - Parasitoid . . . . . . 130
    (6) Flour Beetle Competition . . . . . . . . . 130
  7.4.2 Promising Systems . . . . . . . . . . . . . . 130
    (1) Plant - Herbivore - Exploiters . . . . . . 130
    (2) Predator Competition . . . . . . . . . . . 131
    (3) Mutualism . . . . . . . . . . . . . . . . . 131
    (4) Plant Competition Mediated by Insects . . . 131
    (5) Insect - Pathogen . . . . . . . . . . . . . 131
    (6) Interspecific Competition . . . . . . . . . 132
  7.4.3 Protocol Development (Tribolium Competition) . . 132
    (1) Test Description . . . . . . . . . . . . . 132
    (2) Analysis of Results . . . . . . . . . . . . 133
7.5 Conclusions . . . . . . . . . . . . . . . . . . . . . 134
7.6 References . . . . . . . . . . . . . . . . . . . . . 136

8. METHODS FOR MEASURING EFFECTS OF CHEMICALS ON AQUATIC POPULATION INTERACTIONS . . . . . . . . . . . . . . . . . . . . . 141

8.1 Introduction . . . . . . . . . . . . . . . . . . . . . 143
8.2 Results and Discussion . . . . . . . . . . . . . . . 143
  8.2.1 Evaluations of Test Methods . . . . . . . . . . 143
  8.2.2 Group Discussion . . . . . . . . . . . . . . . 147
    (1) Predation Experiments . . . . . . . . . . . 148
    (2) Competition Experiments and Multispecies Cultures . . . . . . . . . . . . . 152
8.3 Conclusions . . . . . . . . . . . . . . . . . . . . . 155
8.4 References . . . . . . . . . . . . . . . . . . . . . 157

APPENDICES

A. EVALUATIONS OF SELECTED TESTS FOR EFFECTS ON AQUATIC POPULATION INTERACTIONS . . . . . . . . . . . . 161

B. ALPHABETICAL LIST OF PARTICIPANTS . . . . . . . . . . . . 175

## TABLES

Page

4.1 Terrestrial Simulation Models . . . . . . . . . . . . . . . . . . 26
4.2 Forest Succession Models . . . . . . . . . . . . . . . . . . . . 30
4.3 Aquatic Simulation Models . . . . . . . . . . . . . . . . . . . 32
4.4 Examples of Generalized Multipopulation Models . . . . . . . 35
5.1 Evaluation of Major Test Systems for Evaluating Effects of Microbial Processes . . . . . . . . . . . . . . . 56
5.2 Characteristics of Proposed Substrates . . . . . . . . . . . 61
5.3 Number of $CO_2$ Measurements Per Test . . . . . . . . . . . . 62
5.4 Schedule for Sequential Sampling: Aerobic System . . . . . 62
5.5 Parameters Measurable in Terrestrial Model Ecosystems . . . 67
6.1 Model Ecosystems (Lentic Working Group) . . . . . . . . . . 84
6.2 Measurable Ecosystem Properties (Lentic Working Group) . . . . . . . . . . . . . . . . . . . . . . . . . . 85
6.3 Evaluation of Model Ecosystems for Measuring Ecosystem Properties . . . . . . . . . . . . . . . . . . . . 86
6.4 Model Ecosystems (Lotic Working Group) . . . . . . . . . . 88
6.5 Measurable Ecosystem Properties (Lotic Working Group) . . . 89
6.6 Evaluation of Model Ecosystems for Measuring Ecosystem Properties (Lotic Working Group) . . . . . . . . . 90
6.7 Evaluation of Test Systems (Lentic Working Group) . . . . . 91
6.8 Evaluation of Test Systems (Lotic Working Group) . . . . . 94
7.1 Rating and Evaluation of Test Systems for Microbial Population Interactions . . . . . . . . . . . . . . . . . . . 110
7.2 Rating and Evaluation of Test Systems for Plant-Microbe and Plant-Plant Interactions . . . . . . . . . . . . 121
7.3 Ranking and Evaluation of the Proposed Test Systems . . . . 127
7.4 Rating of Arthropod Population Interactions . . . . . . . . . 128
8.1 Evaluation of Predation Tests . . . . . . . . . . . . . . . . 144
8.2 Evaluation of Competition Tests . . . . . . . . . . . . . . . 145
8.3 Evaluation of Multispecies Culture Systems . . . . . . . . . 146

## FIGURES

Page

4.1 Hypothetical Scheme for Selecting Appropriate Models for Use in Hazard Assessments . . . . . . . . . . . . 39
7.1 Typical Survival Curve . . . . . . . . . . . . . . . . . . . . . 116

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# SECTION 1

# INTRODUCTION

This series of six workshops was conducted by the Environmental Sciences Division (ESD), Oak Ridge National Laboratory (ORNL) and sponsored by the Office of Toxic Substances, U.S. Environmental Protection Agency (EPA). The workshops were designed to identify laboratory methods for measuring the ecological effects of chemical substances and to evaluate those methods for their potential utility to the hazard and risk assessment processes of the Toxic Substances Control Act (TSCA). TSCA is comprehensive legislation that subjects the chemical industry in the United States to federal regulation that broadly protects human health and the environment from unreasonable risks resulting from the manufacture, processing, distribution, use, and disposal of a chemical substance. Under TSCA, EPA is responsible for identifying and prescribing test standards to be used in developing the data necessary to predict the risks associated with exposure to chemical substances. Responsibility for implementation of TSCA resides with the Office of Toxic Substances, EPA.

Results from the workshops were used by ESD staff in preparing a critical review of Methods for Ecological Toxicology*. This review was prepared to aid EPA in investigating the potential for developing test protocols that predict the effects of chemical substances on selected ecological parameters that are indicative of interspecific interactions, community dynamics, and ecosystem functions. Streamlined protocols are necessary if consistent results are to be expected among different laboratories. The workshops were primarily used as a mechanism to collect information about research in progress by bringing together investigators presently working with aquatic or terrestrial laboratory test systems.

The workshops were designed under the assumption that a tiered-testing scheme would be the basis for EPA's environmental hazard assessment process. Such a scheme provides for different levels of testing ranging from simple, inexpensive screening tests to higher levels of increasingly complex, definitive tests. Positive results at one level of testing indicate the need to proceed to the next higher level. Participants were asked to identify tests for measuring the ecological effects of chemical substances and to evaluate those tests in terms of their potential usefulness as predictive tools in hazard assessment. In addition, one of the workshops addressed the role of mathematical modeling in ecological hazard assessment and another addressed the major problems associated with assessment and policy requirements of ecological toxicology testing under TSCA.

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*Hammons, A. S. 1981. Methods for Ecological Toxicology. A Critical Review of Laboratory Multispecies Tests. Oak Ridge National Laboratory, Oak Ridge, Tennessee. EPA 560/11-80-026; ORNL 5708.

This report represents a summary of the results of all six workshops. It is obvious that even though an attempt was made to design the workshops as consistently as possible considering the different topics, assigned tasks were, nevertheless, handled differently by the different groups of participants. Workshop results are presented without argument or attempts to include information that was not actually discussed during the workshops.

The criteria that were used in evaluating identified tests were defined as follows:

Cost Per Test. The total cost of completing a test for a single chemical assuming that the facilities are already available.

Documentation. The extent to which the behavior of a laboratory system (not necessarily toxicological) has been investigated and reported.

Generality. The usefulness of the test in predicting the responses of a variety of interspecific interactions or ecosystems and their major components.

Rapidity. The total amount of time required to complete a test assuming that facilities already exist.

Realism. The ability to unambiguously interpret the response of the test system in terms of responses of real ecosystems.

Rejection Standards. Defined criteria for rejecting test results that range from informal or common sense criteria (e.g., many controls die) to a complete and well-defined set of criteria (e.g., more than 10% of controls fail to achieve a weight of 20g).

Replicability. The variance in response within an experiment among individual units of a test system.

Reproducibility. The ability of a test to produce common results in different laboratories.

Sensitivity. The ability of the test to produce measurable responses at low doses of test chemicals.

Social Relevance. The value to society, direct or indirect, of the response measured. The value may be economic, aesthetic, or indirectly related to human health.

Standardization. The definition of conditions and components of a test system to allow different laboratories to obtain similar results from a test.

Statistical Basis. Accepted statistical criteria for detecting and interpreting responses of the test system.

Training, Expertise Requirements. The extent to which use of a test may be limited by requirements for higher education, specialized training, or expertise.

Validity. The extent to which the responses of a test system are known to reflect responses in the field.

# SECTION 2

# CONCLUSIONS AND RECOMMENDATIONS

## 2.1 Introduction

No laboratory systems or mathematical models are presently ready for use as predictive tools in environmental hazard assessment. Nevertheless, there are several promising methods that are recommended for further development. For example, protocols are suggested for testing chemical effects on sediment cores, mixed microbial cultures, model streams, *Tribolium* (flour beetle) competition, and carbon and nitrogen mineralizations. Further experimentation is needed to adapt many of the systems to chemical testing and to standardize and validate all of the proposed protocols.

Selection of appropriate multispecies tests is not easy. Many factors must be considered which require further research before proper choices will be clear. For example, which types of systems will yield the most useful, generalizable information when tested? Which properties are most critical to the functioning of the system and most sensitive to chemical stress? What magnitude of effect is significant? What are the criteria for validating these systems in the field?

Perhaps the major problem to be resolved before interspecific interactions can be useful in hazard assessment is extrapolation or generalization of experimental results to predict effects in natural ecosystems. The degree to which chemical effects may be distorted by the necessary simplification of laboratory test systems is not known. Research is needed to (1) compare the sensitivity of laboratory systems to that of natural ecosystems, (2) relate the ecological complexity of laboratory systems (number of taxa or number of functional groups) to their responses to chemicals, and (3) develop models or other analytical approaches to link laboratory results to predictions about chemical effects in nature.

Although many questions remain unanswered about the proper use of multispecies tests in hazard assessment, certain generalizations can be made. Because these tests generally are more complex than single species tests and the results are more difficult to interpret, they probably will be of most practical use in the later stages of a tiered-testing scheme. As a result, relatively few chemicals may ever be tested in these systems. Chemicals probably will reach the higher levels of testing only if their economic or commercial potential is great enough to justify additional expensive tests when a probable hazard has already been indicated. A testing sequence should begin with single-species screening tests. These tests are needed to (1) flag potential problems, (2) help select additional tests and test organisms, and (3) aid in the interpretation of multispecies test results. Despite the uncertainties associated with the use of multispecies tests, they may be necessary to determine ecological hazards if simpler tests indicate potential problems. Multispecies tests will be especially important if the questionable chemical will be either persistent in the environment or continuously released into the environment. The final step of an entire testing scheme should be field validation.

The main conclusions from this series of workshops are briefly outlined in the following sections.

## 2.2 Terrestrial Test Systems

### 2.2.1 Population Interactions

1. The clover-fescue interference system received the highest rating among plant and microbe systems because it combines interactions between plant populations with interactions between plant and microbial symbionts.

2. Other plant microbe test systems proposed for further development include: mycorrhizae-plant; Rhizobium-legume; wheat-wheat rust; carrot-crown gall; plant-nematode; and agricultural soil microcosm.

3. There is no clear perception that one or a few particular types of arthropod interactions are superior to the others.

4. Tests for chemical effects on arthropod population interactions with the greatest potential for use in hazard assessment are: plant-white fly-parasitoid; corn-earworm-exploiters; alfalfa-aphid-parasitoid; plant-brown scale-exploiters; housefly-blow fly-parasitoid; and flour beetle competition.

5. The potential test systems for microbial population interactions and community properties are not highly recommended.

### 2.2.2 Ecosystem Properties

1. Tests for predicting chemical effects on microorganisms should be as close to the natural system as reasonably possible.

2. Kinetic (sequential) testing should be done.

3. Measurements of effects of general metabolic processes occurring in the whole population or community (e.g., $CO_2$ formation, $O_2$ consumption) are more meaningful than results from more selective tests based on a single enzymatic criterion (e.g., sulfatase, phosphotase, amylase).

4. A test system is proposed to measure the effects of chemicals on carbon and nitrogen mineralizations simultaneously using environmentally relevant high nitrogen substrates and mixed microbe populations that can be manipulated easily by technicians with minimal training.

5. It is unknown whether microorganisms in the soil are sensitive indicators of the effects of chemicals.

6. No model ecosystem, synthetic or excised, is considered ready to serve as a test protocol.

7. Both defined (gnotobiotic) systems and intermediate-sized grassland microcosms are recommended for further development.

8. The relevance of measured parameters to major ecosystem processes should be determined.

9. Encasement materials should be evaluated in terms of leachability, absorptive capacity, optical properties, and durability.

10. The effect of variation in the chemical, physical, and microbiological properties of soil on responses to chemicals should be determined.

11. Round-robin evaluation is needed for all tests.

12. Field validation is necessary for all tests.

## 2.3 Aquatic Test Systems

### 2.3.1 Population Interactions

1. Laboratory systems involving predation, competition, and multiple population interactions are available for development as hazard assessment protocols, but few systems have been used for chemical testing.

2. Comparison of simple and complex laboratory systems with natural systems is a major research priority.

3. Many tests require special facilities or skills.

4. Reproducibility is virtually unknown for all systems evaluated.

5. Relative sensitivities of different laboratory systems is an important research problem.

6. Few tests are rated highly expensive, but absolute cost per test is generally not known.

7. Predator-prey tests appear to be more rapid, more replicable, more advanced, and more readily standardized than competition tests or multispecies culture systems.

8. Predator-prey systems have been used very little to test effects of organic chemicals.

9. Predator-prey tests are more sensitive to chemicals than acute single-species bioassays in many cases.

10. Multispecies tests are more useful in the intermediate stages of hazard assessment.

11. Criteria that should be used in setting up the optimal system for testing predator-prey relationships include criteria for (1) the test organism, (2) the test systems, and (3) the test protocols themselves. (These are outlined in Section 8.2.)

### 2.3.2 Model Ecosystems

1. Extrapolating from laboratory tests to natural systems is the major problem in model ecosystem research.

2. Research is needed to identify the responses most sensitive to chemical stress.

3. A predictive or mimicking model ecosystem is probably most useful in later stages of hazard assessment.

4. A generic ecosystem (e.g., mixed-flask culture) is probably most useful earlier in the testing sequence to screen chemicals for their ability to disrupt ecosystem processes.

5. Smaller systems are more replicable and more easily standardized among laboratories.

6. Statistical analysis of results is easiest with small systems.

7. Standards for rejection are less likely for larger models.

8. Interpretation of chemical effects is more difficult for larger systems.

9. Systems meeting the operational criteria for a screening test are least generalizable to natural ecosystems.

10. Protocols were developed for chemical effects on model streams, mixed microbial cultures, and sediment cores.

11. Each proposed protocol needs extensive refinement and validation.

## 2.4 Mathematical Models

1. Available mathematical models appear to be best suited for use as relatively inexpensive and rapid qualitative tools for preliminary screening to explore the possible effects of chemicals.

2. Considerable development and testing will be required before mathematical models can be reliably used for predicting effects of chemical substances on ecosystems.

3. An overall strategy for selecting and applying models is required before models can be used productively in hazard assessment.

10

# ASSESSMENT AND POLICY REQUIREMENTS OF MULTISPECIES TOXICOLOGY TESTING PROCEDURES

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## SECTION 3

## ASSESSMENT AND POLICY REQUIREMENTS OF MULTISPECIES TOXICOLOGY TESTING PROCEDURES

### 3.1 Introduction

This workshop was designed to help establish guidelines for evaluation of laboratory tests for use in predicting the ecological effects of chemical substances above the population level of biological organization. Three main topics were selected by the Environmental Sciences Division (ESD) staff to be addressed in roundtable discussions during the two-day workshop:

1. What properties and functions of communities and ecosystems should be addressed in evaluating the ecological hazard of a chemical? Consider: Ecological significance, System specificity, Natural variability, Sensitivity to chemical disturbance, Ability to be measured, Ability to be simulated in the laboratory.

2. How should these properties and functions be used in a hazard evaluation process? Consider: Utility in preliminary screening, Utility in predictive modeling, At what stages particular types of information are needed.

3. Identify criteria that are important in evaluating the usefulness of any test. Examples might include: Replicability of test, Sensitivity of test, Statistical basis for interpreting results, Standards for rejecting test results, Frequency of failure, Time required, Cost per chemical.

The following sections summarize the workshop discussions and conclusions. Comments and suggestions that were considered by ESD staff to be most relevant to the goals of the workshop are included. No attempt was made to develop a consensus report.

### 3.2 Results and Discussion

Much remains unknown about the use of multispecies laboratory tests for predicting chemical effects on interspecific interactions and ecosystems. The significance of measurable effects to the health of these systems has generally not been determined. For this reason, emphasis was placed on the basic need for developing generally accepted hypotheses that would allow the identification of significant effects on interspecific interactions or ecosystem properties and that would enable tests to be statistically designed to either validate or disprove previously formulated testable hypotheses. It is important to understand perturbed or stressed systems well enough to select the most important responses to test. So that test results can be properly evaluated, it is equally important to understand the generalities of the mechanisms of processes in non-stressed systems and the ecological significance of variations in measurements. Until such knowledge is available, the safest objective of an ecological hazard evaluation process might be to maintain viable ecosystems close to their present point of balance.

Development of a data base on studies of notable "natural experiments"--ecosystems that have already been impacted by toxic substances released into the environment (e.g., smelters or accidental spills) may be a way to help identify appropriate parameters to test. Such an exercise would be particularly useful if assessment of such studies uncovered patterns of "typical" effects on "typical" ecosytems.

No single species, combination of species, or any ecosystem can be representative of all species or all ecosystems. As a result, the choice of species or systems for a test will necessarily vary and depend heavily on the uses, residues, and resulting exposure potential expected for each chemical under consideration. Some participants recommended that the most sensitive and the most likely to be exposed species or systems would be the best choices to test. Obviously, determining the most sensitive species or system would not be an easy task. In addition, if an ecosystem that would likely be exposed to a questionable chemical contains species of particular economic or aesthetic value, special attention must be given to the possible effects on those particular species. Special attention was also suggested for multispecies interactions associated with pathogens and parasites. For example, hosts weakened by exposure to toxic substances may be more prone to succumb to disease or predatory attack. There was a suggestion that species should also be tested separately to determine whether either might be affected independently. Participants cautioned (1) against assuming that protection of an ecosystem, as determined by gross parameters, would protect all of the individual components of the system, (2) against substituting multispecies tests for single-species tests, and (3) against excluding "special" ecosystems or worst-case systems for consideration in a hazard evaluation process.

Several participants speculated that comparatively few chemicals would ever be tested at the highest level in an ecological hazard evaluation scheme where multispecies tests would perhaps be most appropriate. A chemical will reach the higher levels of testing only if its economical or commercial potential is great enough to justify additional expensive testing even after earlier tests indicate probable hazard. Because tests at this level (i.e., most multispecies tests) will be used on relatively few chemicals, they can generally be more complex, sophisticated, time-consuming, expensive, system-specific, etc., than screening tests. Except, perhaps where tests that require purchase of costly, highly-specialized equipment would not be acceptable for limited use. Although the workshop consensus seemed to be that effects on interspecific interactions and ecosystem processes would probably be tested only at the higher levels of a tiered-testing scheme, this does not prevent the development of multispecies tests for screening purposes.

There appeared to be some agreement that, for purposes of the Toxic Substances Control Act (TSCA), aquatic ecosystems may prove to be generally more relevant to test than terrestrial ecosystems. Aquatic systems, especially those lacking sediments, are likely to be more sensitive than terrestrial systems, which are buffered by soil. Consequently, results from tests on terrestrial systems might cause the hazard to aquatic species to be underestimated.

Despite the numerous problems and uncertainties associated with such an effort, tests for chemical effects on ecosystems and/or interspecific interactions will be necessary to determine ecological hazards if simpler tests indicate potential problems. These tests will be necessary especially if the questionable chemical will be either persistent in the environment or continuously released into the environment. Multispecies tests should be used to enhance our knowledge and predictive capabilities.

## 3.3 Conclusions

### What properties and functions of communities and ecosystems should be addressed in evaluating the ecological hazard of a chemical?

Integrative parameters, such as diversity or total primary production, were generally considered less useful in hazard assessment than information on the presence or absence of particular species in the community. Species composition and interspecific interactions (competition, predation, herbivory, and parasitism) are probably more sensitive to chemicals than gross ecosystem functions (primary production, secondary production, and decomposition) because changes in the latter functions can be compensated by shifts in community composition. On the other hand, changes in species interactions and community composition are probably more ecosystem-specific (i.e., less generalizable) than effects on gross ecosystem functions.

Among the emergent ecosystem properties, nutrient cycling and resistance to additional stress were considered to be currently the most useful to hazard assessment.

The opinion of one participant was that predator-prey interactions are easier to study in the laboratory than in the field, whereas the reverse is true of plant-herbivore interactions.

The following comments are presented as examples of individual thoughts concerning this question.

**J. W. GILLETT**: Only those functions need to be tested that are likely to be more sensitive than any single-species response.

**J. C. RANDOLPH**: Production (P), respiration (R), and P/R ratios are functional attributes that have been shown to have a high level of ecological "significance." P/R data have a certain level of system specificity, mostly between terrestrial and aquatic systems; however, these characteristics are not unique to a wide variety of system types. Sensitivity to chemical disturbance is likely to be extremely variable. Although technically somewhat tedious in some cases, there seem to be neither conceptual nor technological constraints on our ability to measure community respiration and production.

**C. F. COOPER**: In the development of multispecies protocols, it may be advisable to start slowly (i.e., to begin with combinations of two species), perhaps along an induced environmental gradient. One could determine how the two species sort themselves out along that gradient (habitat preference; optimal growth under competition) and then see if the equilibrium is shifted when the system is challenged with the chemical in question.

**W. CHAPPELL**: For the longer term there are areas where research needs to be done to establish whether meaningful measurements could be developed for diversity, connectivity, competition coefficients, etc.

### How should these properties and functions be used in a hazard evaluation process?

Most of the information already discussed will be of secondary importance in hazard assessment and need not be tested unless earlier, simpler tests (such as single-species bioassays) indicate potential problems. Effects on some interspecific interactions might be predictable (via mathematical models) from single-species tests. Gross ecosystem function, nutrient cycling, and resistance to stress could be tested after the initial screening tests if the exposure assessment and screening tests indicate a need for further testing. If ecosystem functions are more sensitive than selected single-species parameters, it may be necessary to develop suitable screening tests for effects on ecosystems.

To a large extent, the sequence of tests will be determined by the availability of simple, inexpensive protocols. However, some aspects of the sequence can be based on scientific reasoning. For example:

1. If a chemical is expected to persist in soils or sediments, tests on nutrient cycling and decomposition are warranted.

2. If different species on a single trophic level vary greatly in their sensitivity to a chemical, effects on interspecific competition should be tested.

3. If a chemical affects plants and animals differently, plant-herbivore interactions should be tested.

The final step of an entire hazard assessment scheme should be field experimentation to validate previous tests and confirm predictions.

We agreed that no tests are ready for use as screening tools in hazard assessment. All tests are presently too complex, too expensive, and too difficult to interpret, and none have been validated. The Oak Ridge National Laboratory (ORNL) soil core microcosms now being tested at the Environmental Protection Agency's (EPA's) Corvallis laboratory are perhaps closest to standardization for routine use. Selected individual comments are presented below.

J. C. RANDOLPH: If, at the community/ecosystem level, some predictable input/output (or dose-response) relationship could be established between exposures to hazardous chemicals and ecosystem properties such as production and respiration, there exists the possibility of using these relationships in routine preliminary screening tests. If we wish to investigate the mechanisms of the ecosystem responses to exposure to hazardous chemicals, much additional research is needed. There seems to be little justification for routinely attempting to monitor for complex ecosystem properties, such as nutrient cycling, when we presently know relatively little about the generalities of the mechanisms of nutrient cycling in nonstressed ecosystems. There is a very wide conceptual and technological gap between single-species acute and chronic tests done under carefully controlled laboratory conditions and truly process-oriented ecosystem analysis that by its very nature must be within the particular ecosystem of interest. Thus, it would seem desirable to go to a third-tier level of hazard evaluation for ecosystem processes only after screening and longer-term, single-species tests have indicated a high probability for some effect on some specific ecosystem property.

C. F. COOPER: The test should be statistically designed to validate or disprove previously formulated testable hypotheses about not just the occurrence but the nature of an effect. Preparation of these hypotheses, based on knowledge about the behavior of similar compounds, behaviors in single-species tests, etc., are likely to be the most important and the most difficult part of the program.

W. CHAPPELL: If one could obtain a field sample (e.g., lake water and/or sediment) or a suitable model and subject it to a dose of the chemical of interest, it might be possible to identify the most sensitive species for later single- and multispecies testing under more controlled conditions. This may or may not work, but it seems worth a try.

F. FISHER: The position of a test in a screening system must depend on the difficulty of the actual test procedure, which is difficult to assess at this time. Field testing may be appropriate at the higher tier.

Identify criteria that are important in evaluating the usefulness of any test.

The following criteria (in alphabetical order) are considered important in evaluating the usefulness of any multispecies test for hazard assessment:

Ability to interpret results unequivocally

Cost of capital equipment

Cost per chemical

Existence of standards for rejecting experimental results (e.g., death of controls)

Frequency of rejection of results

Replicability

Sensitivity (i.e., effects are seen at low chemical concentrations, compared to single-species tests)

Skill or expertise required

Statistical basis for detecting effects

Time required

Wide range of sensitivity to different classes of chemicals and a range of sensitivities within classes.

# MATHEMATICAL MODELS USEFUL IN TOXICITY ASSESSMENT

January 8 and 9, 1980

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## SECTION 4

## MATHEMATICAL MODELS USEFUL IN TOXICITY ASSESSMENT

### 4.1 Introduction

> Four and seven years ago, our Father built Him a model,
> And He built it out of the stuff of the computer,
> And He nurtured it with the stuff of His own soul
> And He breathed life into it--and He thought that it was good.
> And He rested.
> And all the models on the face of the earth--they were fruitful
> And they multipled.
> And now--in this great judgement hall we are called
> Together to evaluate these models--for didn't He say that
> There shall come time when the protection agency shall
> Arise among us and the ghosts of dead models shall
> Congregate in the halls of judgement awaiting redemption.
> Yea, all the models, the lame and the sick, even the
> Poorly documented shall come . . .
> And they shall be judged.
> Be this our task? I beg of you LORD, give us strength
> To know the fat from the lean.
>
> --Invocation by Gordon L. Swartzman
> January 9, 1980

The purpose of this workshop was to identify specific mathematical models and general modeling techniques that could be useful for predicting the effects of toxic substances on ecosystems or multispecies assemblages. The workshop sessions were organized around three major topics:

1. Identification and documentation of models and modeling methods potentially useful for prediction of toxic effects.

2. Development of criteria for evaluating the usefulness of models.

3. Identification of research priorities.

Because of the large number of extant mathematical models, the workshop focused on general types of models, categorized as follows:

1. Terrestrial simulation models

2. Aquatic simulation models

3. Generalized multipopulation models

4. Alternative methodologies

Participants were divided into four groups, one for each of these categories. To stimulate discussion, the compositions of the working groups were varied among the three sessions. The criteria and research priorities proposed by the working groups were then presented and discussed at general roundtable sessions. Group participants are identified for each session as follows:

SESSION I (Model Description)

| Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|
| Mulholland | Lorenzen | DeAngelis | Richey |
| Shugart | Hill | Swartzman | Levins |
| Emanuel | Lassiter | Hallam | Goodman |
| Gardner | Park | O'Neill | Rust |

SESSION II (Model Evaluation)

| Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|
| Mulholland | Lassiter | DeAngelis | Levins |
| Gardner | Park | Hallam | Hill |
| Swartzman | Richey | Emanuel | Shugart |
| Goodman | O'Neill | Lorenzen | Rust |

SESSION III (Research Priorities)

Same as Session I

## 4.2 Results and Discussion

We agreed that, although no specific model has proven valuable in predicting the effects of chemical substances on ecosystems, a great many models have potential value.

Ten distinct types of terrestrial simulation models were identified. These types of models ranged in scale from models of single plants (suitable for coupling to more complex models) to models of regional and global biogeochemical cycling. The majority of these models were developed to simulate material transport and cycling and are, therefore, more suitable for predicting transport and accumulation of chemical substances than for predicting their effects on ecosystems. Most could, however, be modified (with varying degrees of difficulty) to incorporate toxic effects.

The many extant aquatic simulation models were divided into three basic types: fate, effects, and traditional ecosystem models. The fate models simulate biotic and abiotic transport phenomena; the effects and traditional ecosystem models simulate ecosystem dynamics. The prediction of effects of toxic substances on ecosystems requires the coupling of fate models to ecosystem models; effects models are ecosystem models expressly designed for this purpose. Like terrestrial models, aquatic simulation models vary widely in scale and complexity. Models have been developed for most types of aquatic ecosystems, including lakes, rivers, estuaries, and seas.

Generalized multispecies models are much less complex than terrestrial and aquatic simulation models. They are not site specific or even ecosystem specific. The structures of these models (i.e., identification of variables, functional complexity, and environmental coupling) can be tailored to suit the objectives of the modeler.

The alternative methodologies category includes all modeling techniques that cannot be placed into one of the other categories. Four alternative methodologies were identified, all distinctly different in approach and application than the ecosystem simulation and generalized multispecies models. These alternative methodologies can be used for such purposes as predicting the effects of stress on the stability of systems, predicting the direction of change of arbitrarily defined variables (e.g., population sizes or production rates) in response to stress, predicting changes in patterns of nutrient cycling, and predicting the evolution of populations subjected to stress. None of these methodologies have yet been applied in chemical effects studies, but all have potential applications.

A variety of criteria were proposed for evaluating the usefulness of models. Some are very general and apply to all model types. Among these criteria are the match between the properties of the model and the objectives of the assessment, the generality and ease of validation of the model, temporal scale and resolution, whether the model makes socially relevant predictions, and whether the model suggests practical monitoring protocols.

Research priorities for both model development and testing were suggested. Development of "standard" models and "standard" environments was proposed for both aquatic and terrestrial simulation models. Development of parameter structure handbooks and flowchart decision trees were recommended as aids in the development of new models and as guides for the selection and use of models in chemical hazard assessment. Theoretical studies were also recommended to delimit the possible effects of chemicals on ecosystems. More generally, in addition to the development of specific models, an overall strategy for using models as part of hazard assessments should be developed. This strategy should include modification of current laboratory protocols to provide appropriate data for model input, the development of evaluation criteria and benchmark test data, and verification of model predictions by comparison to field monitoring data.

Models and modeling methods identified by the workshop participants as being potentially useful in chemical hazard assessment are briefly described in the following sections. These discussions focus on the properties that are most relevant for evaluating the usefulness of the models in hazard assessment. Among these are:

1. Spatial and temporal scale. Spatial scale relates to the size of the region being modeled (e.g., local site, watershed, state, or whole planet). Temporal scale relates to the time period being modeled (days, weeks, years, or decades). It is important that the spatial and temporal scales of the model be commensurate with the spatial and temporal scales of the expected impact.

2. Trophic complexity. In most ecosystem models, the hundreds or thousands of individual species of organisms are aggregated into groups of species with similar properties (e.g., trophic levels or functional groups). Alternatively, in some models the organisms of interest (e.g., tree species in forest succession models) are modeled as individual species, and the remainder are aggregated into broad groupings or ignored completely. Clearly, the way in which species are aggregated affects the purposes for which a model can be used.

3. Mathematical formalism and computer implementation. What kind of equations are used (e.g., differential equations or finite difference equations; deterministic or stochastic equations; linear or non-linear equations)? What programming language is used to translate the equations into a computer program? Is the program documented so that it can be understood and used by persons other than the program developers? Are program modifications required to run the program on different computers? The mathematical formalism used in a model affects the kinds of purposes for which it can be used and, secondarily, the cost and difficulty of using it. In addition, programming considerations can place severe constraints on the usefulness of complex simulation models. An otherwise suitable model can be virtually useless if it can be understood and used only by its creators or if it can be run on only one kind of computer. The ideal simulation model should be documented in a user's manual that describes the computer program and explains how to use it. The program should be written in a programming language, such as FORTRAN, that is available on most computers.

4. Kinds of effects predicted. These can include changes in the abundance of populations or of groups of populations, changes in yield of economically important species or changes in the stability of ecosystems.

5. Validation. All models are abstractions and simplifications of reality. Therefore, it is necessary to investigate the correspondence between the properties of the model and the properties of the real system being modeled. Validation must not be confused with verification (i.e., the demonstration that the computer program is an accurate translation of the model's equations) or calibration (i.e., the adjustment of model parameters so that the output of the model matches a data set). Comparisons between model predictions and empirical data (especially experimental data) are particularly valuable for assessing the validity of models to be used in chemical hazard assessment.

6. Original purpose of the model. The purpose for which a model is developed inevitably affects the structure of the model and constrains the ways that it can be used. A model specifically designed for predicting effects of chemical substances will generally be more useful for chemical hazard assessment than will a similar model designed for some other purpose.

7. Modifications needed to predict effects of chemical substances. Models developed for purposes other than the prediction of effects of chemical substances may require substantial modification to be useful in chemical hazard assessment. For example, models designed to predict the bioaccumulation of pesticides or radionuclides generally employ extremely simplistic representations of biological interactions that must be made more realistic to predict biological effects of chemical stress.

### 4.2.1 Terrestrial Simulation Models

The working group on terrestrial simulation models identified ten types of such models and prepared a table (Table 4.1) presenting summary descriptions of each type. In addition, the group developed more detailed descriptions of the five types with which they were most familiar and evaluated each one of these with respect to the criteria presented in Section 4.4.1. These descriptions, along with citations to specific examples of each type of model, are presented below. Readers who are unfamiliar with the principles of simulation modeling and the somewhat specialized language used by modelers may wish to consult the excellent non-technical discussion of modeling written by Walters (1971).

TABLE 4.1. TERRESTRIAL SIMULATION MODELS

| Model type | Spatial/temporal scales | Mathematical structure | Examples | Validation | Applications | Potential uses and needed modifications |
|---|---|---|---|---|---|---|
| Global biogeochemical models | Global/decades to centuries | Mass balance with compartment structure. Linear ordinary differential equations with selected nonlinear terms where necessary. Partial differential equations may be used for circulation in deep ocean. | Woodwell et al. 1971<br>Emanuel et al. 1980a<br>Emanuel et al. 1980b | DDT model has been validated against DDT conc. in deep ocean fishes. In general, models of this type can be tested against atmospheric carbon measurements, with isotope releases associated with the atomic age, and by anecdotal observations. | Global carbon model being used to assess and predict $CO_2$ levels in atmosphere. DDT model has been used in court case. | Can be used to predict global fate of materials with long biological/ecological lifetimes produced in large amounts. Any gas or aerosol (e.g., fluorocarbons) should be considered. Models require that chemicals have analogies with major elements (C,S,N,P). Validation of models of this scale is an area that is in need of investigation. |
| Regional biogeochemical models | Regional (state, river basin, biome)/one to tens of years | Mass balance (same as for global models). | Harrison et al. 1970<br>Hett and O'Neill 1974<br>O'Neill et al. 1972 | Example model used to calculate accumulation in indicator species plus strong arguments on the parts of the models conforming to the known behavior of DDT. In general, validation same as for global models. | DDT court case (see Science 174:1108). Particularly valuable for agricultural products. | Regional scale models are not well formulated, and validation procedures are in an embryonic state. |
| Radiological cycling and transport models | Subregion-km$^2$ to Ha (ecosystems, watersheds, airsheds) | Steady-state solutions of linear ordinary differential equations, with some more elaborate structure. | AIRDOSE, TERMOD, and other models for radiological dose to man.<br>Killough and McKay, 1976<br>Waide and Webster 1976<br>Shugart et al. 1978 | Validated against isotope tracer data. Considerable research on parameter measurement has been completed. | Used in setting NRC standards and in developing, engineering, and regulating power plants. | Great potential in evaluating relatively local accumulation near large facilities. Linear models assume toxic effects are not occurring. Improvements in theory are needed. Interactions with multiple sources and regions not sufficiently considered. |

TABLE 4.1 (continued)

| Model type | Spatial/temporal scales | Mathematical structure | Examples | Validation | Applications | Potential uses and needed modifications |
|---|---|---|---|---|---|---|
| Theoretical ecosystem and community models | N/A | Usually nonlinear ordinary differential equations. | O'Neill 1976<br>DeAngelis et al. 1975<br>Levins 1968<br>Patten et al. 1976<br>Kercher and Shugart 1975 | Empirical testing of hypotheses suggested by theory. | May be useful in testing for trends or directions of impact. | Generally difficult to extrapolate to real systems. Useful in formulating research strategies. |
| Forest succession models | Ha/years to centuries | Stochastic nonlinear differential equations set up for simulation. | Botkin et al. 1972<br>Shugart and West 1976<br>Shugart and West 1980 | Tested against data on chestnut blight or other large perturbation; 20,000 year paleoclimate reconstruction of vegetation; independent data on biomass dynamics. | Used to study effects of species-level responses to $SO_x$ on forest ecosystems. | Predicts socially relevant effects (e.g., changes in timber yield). Used for managing endangered animal species by predicting habitat change. Need to expand to ecosystem level and add more detail on abiotic compartments. |
| IBP Biome models | Subregion, but calibrated from site-specific data/daily, weekly to yearly. | Nonlinear ordinary differential or difference equations. Generally set up as large code simulation models. | Innis 1972 | Usually tested against state variables not used for calibration. Independent tests uncommon. | Can be used for detailed studies that consider the importance of particular processes or system components if sufficient data for calibration can be assembled. | Principally developed to synthesize detailed information on processes, community interactions, etc. Most very complex. Data for calibration likely to limit usefulness. Would be difficult to apply with toxic substance rather than biomass or major nutrients as originally designed. |
| Multistate crop-pest models | Crop sample unit/ week | Stochastic Box-Jenkins ARMA models. Regression. | Hacker 1978<br>Gutierrez 1979 |  | Used in state and local agricultural decision making. Used to develop integrated pest management schemes. | Great potential use. Problem is adding mechanism to a fundamentally empirical representation of population dynamics. |

TABLE 4.1 (continued)

| Model type | Spatial/temporal scales | Mathematical structure | Examples | Validation | Applications | Potential uses and needed modifications |
|---|---|---|---|---|---|---|
| Forestry management models | Ha/1 year to rotation | Stochastic nonlinear difference equations set up for simulation. | Mitchell 1975<br>Arney 1972 | Tested against elaborate data sets. | Used by forestry industry to determine spacing, harvest, and yield. | Predicts socially relevant effects. Models are highly dependent on empirical data. Addition of mechanism is a problem area. |
| Single-plant models. | Single plant/ hours to year | Ordinary differential equations; also electrical analogs. | Dixon et al. 1978 | Tested against elaborate observational and experimental data. | Infer effects of crop strategies: spacing, hybrids, etc. Can be coupled to larger scale models. | Predicts socially relevant effects. |
| Input-output models | Watersheds-Ha to Km$^2$/weeks to annual | Linear algebra - nondynamic. | Lettenmaier and Richey 1978 | Tested against data similar to that used for calibration. | Accumulation estimates. | Static nature of models limits applicability under large perturbations. |

(1) Regional biogeochemical models. These models were developed for use in legal proceedings related to the regulation of DDT. They are regional in scale, with all biotic components aggregated into trophic levels. All components of the ecosystem, from abiotic compartments through top carnivores, are included. The models are formulated as differential equations describing mass balance; the number of state variables can vary between 3 and about 15. All are coded in FORTRAN. No user's manuals exist. Examples of these models can be found in Harrison et al. (1970) and Hett and O'Neill (1974).

Regional biogeochemical models have been used to predict accumulation of DDT in biotic compartments, especially in top carnivores. They can also predict toxic effects of DDT. Although it would be difficult to modify these models to predict effects of chemical substances other than DDT, the same principles and ideas could be useful in formulating new models for those substances. Regional DDT cycling models are relatively low in generality, require relatively large amounts of data for calibration, and are difficult to validate. However, they are comparatively easy to use, and their temporal scales match the temporal impact scale (years to decades) for chemical substances.

(2) Radiological cycling and transport models. These models were developed as hazard assessment tools for isotope releases. They are airshed models for food chains and are used to predict the food chain transport of isotopes from airborne dispersion from a point source. As a result, the probable dose to humans can be predicted. Chemical effects could not be predicted without substantial model modifications.

Large amounts of data are required. If sufficient data are available, they can be readily validated. Socially relevant predictions are made, such as the dose to man resulting from the release of a chemical substance from a point source. However, the time scales of these models do not match basic impact scales for toxic substances. The models are multiplicative chain models. State variables are concentration ratios, rates of release, and decay rates. FORTRAN is the programming language used. User's manuals and interactive codes are available and descriptions of the models have been published in the open literature (e.g., Hoffman et al. 1977; Killough and McKay 1976; Schaeffer et al. 1978).

(3) Global biogeochemical models. These models were designed to predict the $CO_2$ concentration in the atmosphere resulting from combustion of fossil fuels. Some have been adapted for modeling global cycling of DDT (Woodwell et al. 1971).

The models used coupled differential equations that are usually linear, but have selected nonlinear terms. FORTRAN is usually the programming language used; however, a few models are in simulation languages such as CSMP (e.g., Gowdy et al. 1975). In most cases, documentation is sketchy, although all are described in journal articles (e.g., Killough 1980; Emanuel et al. 1980a and b; Gowdy et al. 1975; Bacastow and Kealing 1975; Bjakstrom 1979). These models are modified frequently.

Extensive modification would be required to handle cycling of toxicants. The cycle must be analagous to that of major chemical elements material inputs. They cannot predict the effects of chemical substances nor do they predict socially relevant impacts. Moderate amounts of data are required. Validation is relatively easy.

(4) Forest succession models. All these models simulate forest succession over long time scales. They model soil compartments and vegetation and predict the effects of $SO_2$, climate change, and species deletion on forest succession. These models were developed as a research aid to understanding ecosystems; they are formulated in terms of stochastic nonlinear difference equations and are coded in ANSI Standard FORTRAN. Most of the succession models cited in Table 4.2 are documented in the open literature.

TABLE 4.2 FOREST SUCCESSION MODELS

| Name | Forest type | Citation |
|---|---|---|
| JABOWA | Northern Hardwood Forest | Botkin et al. 1972 |
| FORET | Southern Hardwood Forest | Shugart and West 1976 |
| BRIND | Australian Eucalypt Forest | Shugart and Noble 1980 |
| FORMIS | Floodplain Forest | Tharp 1978 |
| SELVA | Puerto Rican Rain Forest | Dolye et al. 1981 |
| FORAR | Mixed Oak-Pine Forest | Mielke et al. 1978 |
| KIAMBRAM | Complex Notophyll Vine Forest | Shugart et al. 1980 |

No model modifications are required to predict the effects of chemical substances. The temporal scales modeled are applicable for either long-lived chemicals or for continual release of short-lived chemicals. The predictions, such as changes in timber production, made by these models are obviously socially relevant.

(5) Theoretical ecosystem model. DeAngelis et al. (1975) developed a generalized model for use in theoretical studies of ecosystem structure and function. The model can be applied to any type of ecosystem. Nonlinear algebraic equations are used; these can also be used as terms in differential equations (O'Neill 1976). The functions are completely documented in the journal article cited above. No programming is required for steady-state analysis of simple models. Analysis by numerical simulation would require writing a program.

This model has never been used to predict effects of toxic substances on a real ecosystem and would require extensive modification to predict such effects. This model cannot be easily validated, does not make socially relevant predictions, and does not suggest a monitoring protocol. Like the similar models described in Section 4.2.3, this model appears to be useful primarily in formulating research strategies and in initial screening studies.

### 4.2.2 Aquatic Simulation Models

The working group on aquatic simulation models identified three types of such models: ecosystem models, fate models, and fate-and-effects models. Ecosystem models focus on biological processes, such as primary production, grazing, predation, and decomposition. Physical and chemical interactions and transport phenomena are either ignored or treated superficially. Ecosystem models can predict the biological effects of chemical substances, but cannot predict the movement and fate of chemicals. Conversely, fate models emphasize physical and chemical interactions and transport phenomena at the expense of biological realism. Fate models can predict the movement, chemical transformations, and fate (including bioaccumulation) of chemical substances, but cannot predict biological effects. Recently, efforts have been made to develop hybrid models, here called fate-and-effects models, that can predict both the fate and biological effects of chemical substances in aquatic ecosystems.

(1) Ecosystem models. Aquatic ecosystem models exist for entire lakes, streams, estuaries, or open seas (see Table 4.3). The original purposes for developing these models are varied. For example, some were constructed for research purposes, some for predicting the effects of eutrophication (e.g., Chen and Orlob 1975), and one (Tetra Tech, Inc. 1979) for predicting the effects of power plants. Although none of these models have been used to predict the effects of toxic substances on ecosystems, all are detailed enough for the effects of toxic substances on organismal physiology to be extrapolated to population and ecosystem effects.

The level of aggregation for these models varies; some are aggregated into trophic levels, and some into two or more functional groups within trophic levels. In general, the lower trophic levels are modeled in the greatest detail, although most models include levels through piscivorous fish.

TABLE 4.3. AQUATIC SIMULATION MODELS

| Model type | Spatial/temporal scales | Mathematical structure | Examples | Validation | Applications | Potential uses and needed modifications |
|---|---|---|---|---|---|---|
| Ecosystem | Whole ecosystem (stream, lake, estuary, coastal upwelling, open sea)/days to years | Generally nonlinear differential equations. | Scavia et al. 1976<br>Steele and Frost 1977<br>Park et al. 1974 | Calibration using one data set followed by testing against an independent data set. | Most used only for research. Some used to predict effects of eutrophication or power plants. | Can be used to predict effects of toxic substances on trophic levels or functional groups. Most better suited to predicting effects on lower trophic levels than on fish. All must be coupled to fate models. Research on error propagation in these models is needed, especially for higher trophic levels. |
| Fate | Whole ecosystem/ hours to years | Linear or nonlinear, ordinary or partial differential equations. | Smith et al. 1977<br>Mogenson and Jorgensen 1979<br>Fagerstrom and Asell 1973 | Comparison between distribution of tracer predicted and observed distribution from field or microcosm data. | Used to predict transport and fate of pesticides and heavy metals. | Must be coupled to ecosystem models. |
| Fate-and-effects | Whole ecosystem/ days to years | Nonlinear differential equations. | Falco and Mulkey 1976 | Same methods as for ecosystem and fate models. Existing effects models have not been validated. | None have been applied. | Potentially useful for predicting transport, fate, and effects of toxic substances, but much further development and testing is needed. Improved data bases are also needed. |

Most of these models use nonlinear differential or difference equations describing mass balance. FORTRAN is the program language used most frequently. Machine dependency varies. In general, model documentation is very good (e.g., Scavia et al. 1976; Steel and Frost 1977; Anderson and Ursin 1977). User's manuals are available for most models listed.

As would be expected, model validation was considered easier to achieve for lower trophic levels and abiotic compartments than for fish.

(2) Fate models. Fate models are available for lakes, streams, and estuaries (Table 4.3). These models were constructed to predict the transport and fate of pesticides or other chemical substances. Bioaccumulation is also included. The level of aggregation in fate models usually includes abiotic campartments plus trophic levels, although a few, such as PEST (Park et al., unpublished draft), model functional groups within trophic levels. The abiotic compartments are usually modeled in detail. Biotic compartments include primary producers such as phytoplankton and/or macrophytes and primary consumers such as zooplankton and/or benthos. Fish may or may not be included.

These models use nonlinear or linear differential equations. Sometimes partial differential equations are used to simulate two-dimensional hydrodynamics. As with ecosystem models, the program language most frequently used is FORTRAN. In general, documentation of these models is not as good as that for ecosystem models, although the EXAMS (Lassiter et al. 1978) and PEST (Park et al., unpublished draft) models are reasonably well documented. Documentation for the European models (Fagerstrom and Asell 1973; Mogenson and Jorgensen 1979) is not known.

These models cannot predict the effects of chemical substances on ecosystems, but must be coupled to ecosystem models. PEST, for example, is compatible with CLEANER (Park et al. 1974).

(3) Fate-and-effects models. Efforts are now being made to develop models that predict the tranport, fate, and effects of toxic substances in aquatic ecosystems. For example, Falco and Mulkey (1976) have described a pesticide fate-and-effects model. Falco and Mulkey coupled a one-dimensional fate model (used to predict the movement and transformation of Malathion® in a river) to a simple dose-response model that predicts reductions in standing crop of fish caused by exposure to Malathion®. The model consists of linear and nonlinear ordinary and partial differential equations, and is coded in FORTRAN. No user's manual is available. Although fate-and-effects models are specifically designed to predict the effects of chemical substances on aquatic biota, none have been used in practical applications to date.

### 4.2.3 Generalized Multipopulation Models

Ecosystem simulation models are intended to be realistic representations of particular ecosystem types. Modifying them to model a different ecosystem can be time-consuming and expensive. Alternately, it is possible to construct simple, highly generalized multipopulation models that can be rapidly and inexpensively tailored to fit any system of interacting populations, aquatic or terrestrial. Using this modeling strategy, no attempt is made to model every component of an ecosystem; only those processes believed to be critically important are modeled. Transport phenomena are not incorporated in these models. Thus, the models can be used to predict the effects of chemical substances on systems, but not the fate of those substances. These models are not thought to be appropriate for detailed chemical and site-specific assessments; however, they can be used in the early stages of a hazard assessment to rapidly explore the possible effects of chemical substances. Results of such studies can aid in determining whether a more detailed (i.e., expensive and time-consuming) modeling effort is warranted.

Four categories of generalized multipopulation models were identified. In order of increasing complexity, these are:

Functionally simple, not environmentally coupled.
Functionally simple, environmentally coupled.
Functionally complex, not environmentally coupled.
Functionally complex, environmentally coupled.

Within each category, models can be either spatially homogeneous or spatially complex and either age-dependent or not. Table 4.4 lists some examples of most of the categories. Although many of these examples were developed with particular systems of populations in mind, the principles employed can be applied to other systems as well.

### 4.2.4 Alternative Methodologies

For the purposes of this workshop, "alternative methodologies" were defined as any modeling technique that does not fit into one of the other three categories. Four such techniques were identified. Two of these, loop analysis and time-series averaging, are methods of analyzing the qualitative behavior of systems of coupled differential equations. They could be applied to many of the generalized multipopulation models discussed in Section 4.2.3. Input-output analysis is a method of econometric analysis that has been modified for use in analyzing material budgets in ecosystems. Natural selection models are applications of population genetics theory to the problem of predicting the evolutionary response of populations to toxic substances.

(1) Loop analysis. This modeling technique is designed to analyze partially specified systems, i.e., systems in which the

TABLE 4.4 EXAMPLES OF GENERALIZED MULTIPOPULATION MODELS

| Type[a] | Age structure[b] | Spatial structure[b] | Reference |
|---|---|---|---|
| 1 | - | - | DeAngelis et al. 1975;<br>Canale 1970;<br>Rescigno and Richardson 1957 |
| 1 | - | + | Levins 1974 |
| 1 | + | - | Hassell and Comins 1976;<br>Pennycuick et al. 1968 |
| 2 | - | - | Emanuel and Mulholland 1975 |
| 3 | - | - | Hsu et al. 1977 |
| 3 | + | - | Travis et al. 1980 |
| 4 | - | - | Craig et al. 1979 |
| 4 | + | - | Eggers 1975 |
| 4 | + | + | Andersen and Ursin 1977 |

[a] 1 = Functionally simple, not environmentally coupled.
2 = Functionally simple, environmentally coupled.
3 = Functionally complex, not environmentally coupled.
4 = Functionally complex, environmentally coupled.

[b] - = Age structure (or spatial structure) absent.
+ = Age structure (or spatial structure) present.

patterns of interaction among the component variables are known, but parameter values and functional forms are not (Levins 1974; Lane and Levins 1977). The level of aggregation and trophic levels modeled are arbitrary. This type of analysis can predict effects such as local stability, direction of change of variables in response to altered parameter values (e.g., input of chemical substance) and correlations among variables responding to different inputs (Levins 1974; Lane and Levins 1977).

Coupled differential or difference equations are used. The analysis can be validated by comparing the predicted response of a system to a parameter change against the actual response of a perturbed system. This type of analysis has not been used to predict the effect of chemicals. Loop analysis can be used to (a) predict the response of a multipopulation system to an applied stress, (b) identify critical parameters that should be measured, and (c) identify system properties that enhance or reduce impacts. It may not be applicable to systems that are far from equilibrium.

(2) Time averaging. Time averaging can be used to model any system of interacting populations or aggregates of populations (Levins 1979). This methodology, which is complementary to loop analysis, was developed to extend ecological theory to nonequilibrium systems. The kinds of effects predicted are changes in variances and covariances among variables as affected by parameter change.

Coupled differential equations are used. Analysis focuses on statistical moments of variables, especially second-order statistics. Validation can be accomplished by comparing predicted changes in variances and covariances against actual responses of a perturbed system by using pre- and post-perturbation time series. This method has not been used to predict the effects of chemical substances.

Time averaging is potentially useful for analyzing time-varying systems that are far from equilibrium. Time averaging can be used to (a) characterize microcosms before addition of chemical substances, (b) distinguish populations that are directly affected by a chemical substance from those that are indirectly affected, and (c) provide warning about possible structural change caused by chemical substances.

(3) Input-output analysis. Input-output analysis has been used to compare material cycling patterns in different ecosystems (Finn 1976; Hannon 1973; Lettenmaier and Richey 1978). It has been hypothesized that structure and cycling indices derived from input-output analysis might also be useful as indicators of environmental stress. These indices can be computed from a matrix of material flow coefficients using a computer program written in FORTRAN IV. This program can be coupled to "process" models that compute the flow coefficients.

The analysis can be applied either to whole ecosystems or to subsystems within ecosystems. The indices are now useful primarily for descriptive purposes and as indicators of system dysfunction caused by stress. In theory, input-output analysis can be used to predict changes in material flow patterns in response to stress to the biota, but further development and testing are required before it is known whether this is feasible in practice.

(4) Natural selection models. Population biologists have used a variety of models to study the evolution of populations and systems of interacting populations in response to changes in their environments (Levins 1968; Kimura and Ohta 1971). All of these models relate rates of changes in gene or phenotype frequencies to selective pressure, heritability, and genetic variance within populations. They can be used to predict adaptive responses of species to chemical substances and to predict the effects of those responses on population size, location, behavior, and interactions with other species.

Natural selection models can be validated by comparing predicted changes in gene frequencies to actual changes in a population experimentally exposed to stress. Although they have not been used to predict the effects of chemical substances on populations, they are potentially valuable for this purpose because populations in nature frequently evolve in response to exposure to toxic substances. Pesticide tolerance in insects and antibiotic resistance in pathogens are notorious examples. Practical applications would require experimental work to measure the genetic variances in tolerance within and between populations for species of interest and to estimate selection intensities in the field.

## 4.3 Conclusions

We recognize that few, if any, existing models of any kind have been demonstrated to be useful for predicting the effects of chemical substances on ecosystems. Ecosystem simulation models are the only type to have had significant applications to date, but their complexity makes their use comparatively difficult and expensive. Generally, available mathematical models appear to be better suited for use as relatively inexpensive and rapid qualitative tools for preliminary screening to explore the possible effects of chemicals than for use as detailed chemical and site-specific hazard assessments.

### 4.3.1 Criteria for Evaluating and Selecting Models

No single model or model type can fulfill all needs associated with environmental hazard assessment. For this reason, one of the workshop tasks was to develop criteria that could be used to evaluate the usefulness of existing models, modified versions of existing models, and new models. These criteria include not only the properties of the models themselves but also the match between the capabilities and deficiencies of the models and hazard assessment objectives.

Nine criteria for evaluating the usefulness of models were developed:

1. The degree of modification required for handling toxic material inputs. Can chemical inputs be modeled directly? Are the physical and chemical processes that govern the transport and fate of chemical substances included in the model? Are the biological processes directly affected by toxic materials included in the model?

2. Data requirements. Is the amount of data required for parameterizing the model consistent with the available resources (i.e., time and money)?

3. Generality. Can the model be used for only one geographic region or ecosystem type or can it be easily applied to others?

4. Ease of validation. Has the model been validated against baseline data? Are the output variables (i.e., those that must be measured to test the model's predictions) easily measurable? Do modifications required for handling chemical substances invalidate the model? Can the model be tested with microcosm systems as well as with field data?

5. Social relevance. Is the model output relevant to regulatory needs?

6. Relevance to monitoring. Does the model suggest an environmental monitoring protocol? For example, does it suggest indicator variables that are easily measureable and that could be used as early warnings of environmental effects?

7. Spatial/temporal scales. Do the spatial and temporal scales of the model match the basic impact scale?

8. Ease of use. Is the model documentation comprehensible, consistent, and complete? Is the computer code readily available? How much modification is required to implement the code on a different computer system?

9. Acceptance by the scientific community, especially the ecological community. Are these models based on biological ideas and mathematical procedures accepted by most of the ecological community?

Figure 4.1 presents a scheme that could be used to identify specific models for use in hazard assessments; it was prepared using

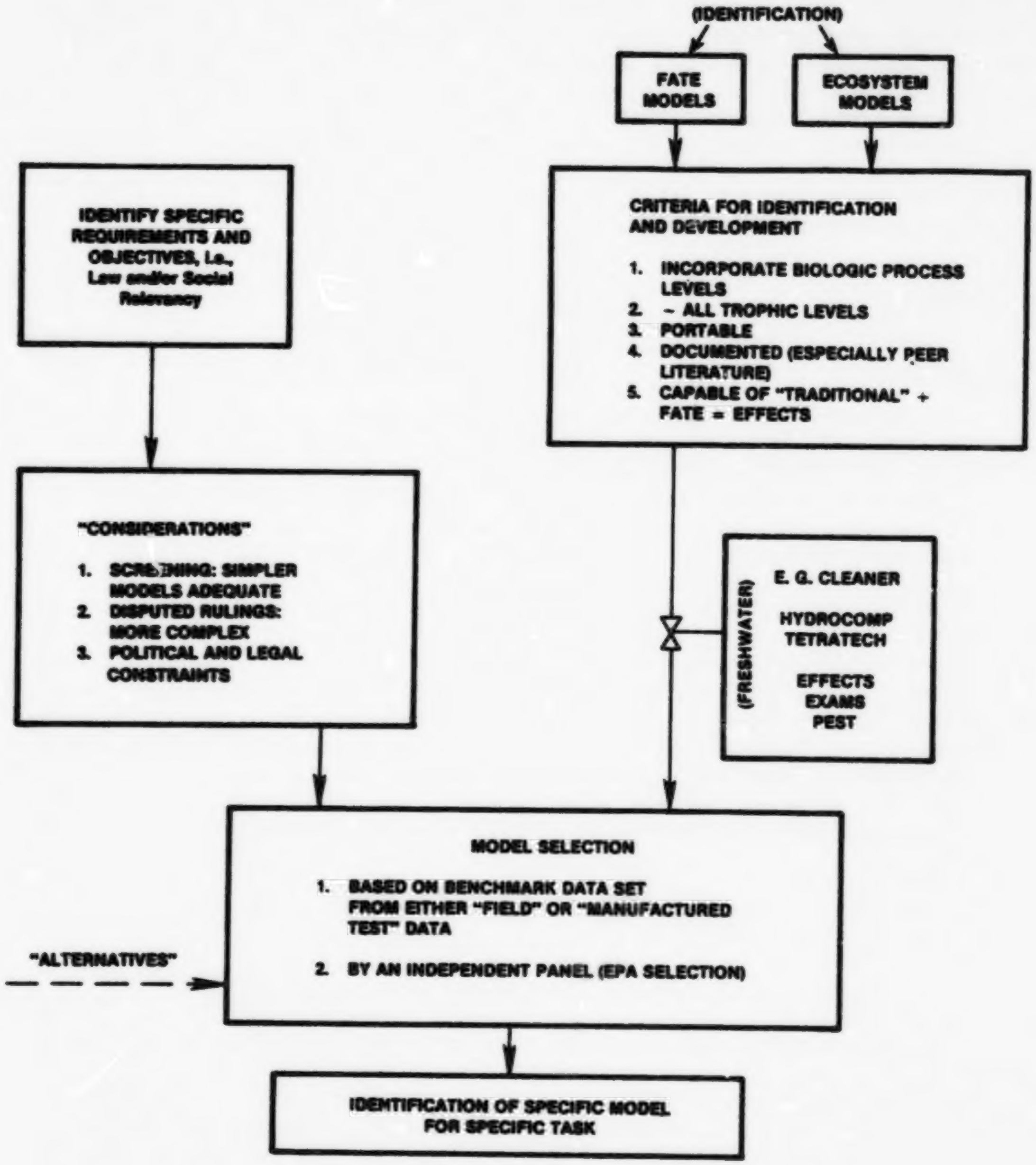
(IDENTIFICATION)
FATE MODELS
ECOSYSTEM MODELS
IDENTIFY SPECIFIC REQUIREMENTS AND OBJECTIVES, i.e., Law and/or Social Relevancy
CRITERIA FOR IDENTIFICATION AND DEVELOPMENT
1. INCORPORATE BIOLOGIC PROCESS LEVELS
2. ~ ALL TROPHIC LEVELS
3. PORTABLE
4. DOCUMENTED (ESPECIALLY PEER LITERATURE)
5. CAPABLE OF "TRADITIONAL" + FATE = EFFECTS
"CONSIDERATIONS"
1. SCREENING: SIMPLER MODELS ADEQUATE
2. DISPUTED RULINGS: MORE COMPLEX
3. POLITICAL AND LEGAL CONSTRAINTS
(FRESHWATER)
E. G. CLEANER
HYDROCOMP
TETRATECH
EFFECTS
EXAMS
PEST
MODEL SELECTION
1. BASED ON BENCHMARK DATA SET FROM EITHER "FIELD" OR "MANUFACTURED TEST" DATA
2. BY AN INDEPENDENT PANEL (EPA SELECTION)
"ALTERNATIVES"
IDENTIFICATION OF SPECIFIC MODEL FOR SPECIFIC TASK

Figure 4.1. Hypothetical scheme for selecting appropriate models for use in hazard assessments.

aquatic simulation models as examples, but it could apply equally to any type of model. The scheme highlights nonmodeling decisions that must be made before appropriate models can be selected for an assessment problem. These include formulating the specific legal or social questions that the model will be expected to answer and specifying whether the purpose of the assessment is the screening of many chemical substances for potential effects or the detailed evaluation of particular substances in connection with regulatory actions.

### 4.3.2 Research Priorities

The workshop participants identified several kinds of research and development activities that are needed to increase the usefulness of mathematical models for predicting the effects of toxic substances on ecosystems.

Further development and testing of ecosystem simulation models is necessary. Improvements are needed in the models themselves and in the data bases used to parameterize and test them. Standard models, specially tailored for the prediction of toxic effects, and standard data sets are needed for representative terrestrial and aquatic environments. As an aid to future model development, we recommend that an ecosystem parameter handbook be compiled. This handbook would include definitions and standard notations for parameters that are used in ecosystem models. The handbook would also include a codification of properties of ecosystems relevant to modeling (e.g., numbers of trophic levels and functional groups in different ecosystem types, relationships between primary and secondary production, and average numbers of prey species fed on by various predators). We also recommend that selected aquatic ecosystem and fate models be coupled to form effects models. The coupled models should then be verified using benchmark data sets. In addition, new methodologies are needed to solve two problems related to fate modeling. First, regional mass balance models are needed to quantify the movement of chemical substances between aquatic ecosystems. Second, specific methodologies are needed to project loadings of important substances.

Theoretical studies using generalized multipopulation models and alternative methodologies should be performed to define the possible responses of systems to chemical substances. Examples of the kinds of results that could be obtained are the identification of (a) system properties that confer resilience or vulnerability to chemical substances, and (b) conditions under which sublethal exposures to chemical substances can cause destabilization of competitive or predator-prey systems. Results of such studies, which can be conducted relatively rapidly and inexpensively, would suggest processes that should be incorporated in more complex models and hypotheses that should be tested using ecosystem simulation models, microcosm studies, and field studies.

Regardless of how many and what kinds of models are available, an overall strategy for selecting and applying models will be required to use models productively as part of the hazard assessment process. This strategy should include the development of flow chart decision-trees for selecting the best model(s) for any given assessment problem. It should also include modifications of current laboratory protocols to provide appropriate input data and the systematic testing of models using microcosm and field data.

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# METHODS FOR MEASURING
# EFFECTS OF CHEMICALS ON TERRESTRIAL
# ECOSYSTEM PROPERTIES

January 15 and 16, 1980

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# SECTION 5

# METHODS FOR MEASURING EFFECTS OF CHEMICALS ON TERRESTRIAL ECOSYSTEM PROPERTIES

## 5.1 Introduction

This workshop considered the availability and utility of multispecies laboratory systems to display the responses of ecosystem level properties to toxic chemicals. The workshop was divided into two working groups. Working group A discussed soil-microbe laboratory systems and microbial processes. Working group B discussed model ecosystems (i.e., systems that contain multiple trophic levels and some diversity in physical structure). Three tasks were assigned to each working group:

1. Identify laboratory microbial systems or model ecosystems that display community and ecosystem level properties and organize them into categories. These categories should be sufficiently distinct to permit generalization about their potential utility in toxicity testing.

2. Evaluate the test systems in terms of the following criteria:

   Replicability

   Standardization (potential for interlaboratory transfer)

   Sensitivity

   Generality (to what range of ecosystems can the results be applied?)

   Equivocality of results

   Statistical bases for interpreting results

   Standards for rejecting results

   Frequency of failure

   Level of training and expertise required

   Time required

Cost per chemical

Appropriate positive controls

Other

3. Consider how these systems could be developed and applied to toxicity testing. For example,

   What test systems are sufficiently developed to serve as test protocols?

   What test systems require further development?

   What types of development are needed: Development of techniques, interlaboratory round robins (validation), field verification, etc.?

   What types of modeling or analysis would be necessary to relate test results to the real world, to generalize the results to a variety of environments or to relate the parameters measured to socially significant parameters?

   What simpler tests could be confirmed by the results of this multispecies test? What more complex test might confirm the results of this multispecies test?

The following sections present the results and discussions of the two working groups. Although these sections are based primarily on written material produced during the workshop by participants, they have been expanded on the basis of notes and recordings from the discussions.

## 5.2 Results and Discussion

### 5.2.1 Microbial Processes

Working group A rewiewed laboratory testing and evaluation procedures for assessing effects of chemicals on terrestrial microbial processes. Basic microbial processes such as carbon, nitrogen, and sulfur transformations are common to the nutrient cycles of all ecosystems. Disruption or promotion of these processes may have ecologically and socially significant impacts.

These discussions emphasized development of a balance between proposing test methods that are practical to perform and ecologically meaningful. The degree of environmental testing required to assess the hazard of any chemical depends on its production, distribution, use, disposal, and fate in the environment. Because of the large number of chemicals produced and the requirement of the Toxic Substances Control Act (TSCA) for cost effective testing, a tiered system involving different levels of testing complexity may be required. Likewise, it is difficult to develop one test method to accommodate the wide range of physical and chemical properties of chemicals that need to be tested in the terrestrial environment. For example, insoluble compounds may require a different set of test conditions than their water soluble counterparts. In addition, the binding behavior of chemicals in soil may play an important role in determining observed toxic effects. Effects may not be observed until the binding capacity of a soil is exceeded or the bound chemical is released into solution by exchange processes. An understanding of various physical and chemical properties of a chemical, as well as its binding properties, is crucial to determining possible toxic effects in the environment.

(1) Identification and evaluation of test systems. The general criteria discussed in Section 5.1 were used to evaluate the methods currently proposed by the EPA for measuring effects on cellulose decomposition and nitrogen and sulfur transformations under TSCA (U.S. EPA 1979). Other existing test systems were also evaluated and an alternate test was proposed by workshop participants. These tests are discussed in the following sections.

(a) Cellulose decomposition. The cellulose decomposition test proposed by the EPA employs the measurement of $CO_2$ formation from an axenic culture of *Trichoderma longibrachiatum* growing on cellulose as a test substrate to determine effects on microbial carbon mineralization. Because this test uses a pure culture and a well defined substrate under controlled laboratory conditions, there are apparent advantages in the potential reproducibility of this method from laboratory to laboratory. In addition, such techniques have been well studied.

Some disadvantages of the proposed cellulose decomposition test are:

1. With an insoluble powdered substrate, degradation of the substrate may be limited by surface area and mixing.

2. The appropriateness of cellulose as a model carbonaceous substrate is quite dubious. Although cellulose is a major component of both plant litter and soil organic matter, it normally occurs in a "masked" form as an intimate physical and chemical association with lignin.

   This lignocellulose complex is much less susceptible to microbial metabolism than purified cellulose itself.

3. *Trichoderma* may or may not be the major organism performing cellulose degradation in soil.

4. Although a single organism test may be very sensitive to specific chemicals, its sensitivity or responses would probably not be representative of the general microbial community.

(b) Nitrogen transformation. The EPA proposed method for testing the effects of new chemicals on nitrogen transformation in the soil measures the production of ammonia from the microbial decomposition of urea. The advantages of this test are that natural, multispecies soil communities are used, instead of pure cultures, as recommended in the cellulose decomposition and sulfate reduction tests. However, we believe that urea is not an ideal choice of organic N substrate. Extracellular soil urease activity can be quite high and can persist in soils in which microbial activity has been eliminated. Thus, ammonification of urea can continue after ureolytic microorganisms have been inhibited by a toxic substance. In other words, the production of ammonia from urea is not a growth-related process and, thus, may be unaffected by a toxic chemical. A second criticism is that an enrichment for ureolytic microorganisms may occur with time and these microorganisms may differ in their tolerance to the test substances when compared to the indigenous microflora in soil not amended with urea.

Furthermore, ammonia may be oxidized to nitrite or nitrate, making interpretation of results, based solely on the measurement of ammonia, impossible.

(c) Sulfur transformation. The method proposed by the EPA for determining possible effects on microorganisms in the sulfur cycle involves a semi-quantitative measure of $H_2S$ production by a pure culture of *Desulfovibrio desulfuricans* in the presence and absence of a test substance. Sulfate reduction is a major process only in anaerobic zones of flooded soils and sediments. In most soil systems, sulfate reduction has only minimal effects on plant productivity (Brock 1966; Russel and Russel 1950).

The proposed method for measuring sulfate reduction is non-quantitative and is based on a single enzymatic oxidation-reduction activity in a single microorganism. This method largely ignores the complex interactions and diversity that can occur in natural microbial populations. It is doubtful that the results of this technique can possibly be extrapolated to the environment. Furthermore, some microorganisms can convert organic sulfur to sulfate directly without the intermediate production of $H_2S$ (Alexander 1961).

Although more expensive and time consuming, a more accurate approach would be to use $^{35}S$ to determine possible effects on the major steps of the S cycle. Such radioisotope methods have been applied to both the soil and sediment system (for review see Brock 1966).

(d) Other existing test systems. Many experimental techniques are used to measure specific or general microbial activities in soils and sediments. A summary of the advantages and disadvantages of many of these methods is provided in Table 5.1. A numerical forced-choice rating system (0 to 4) was employed to estimate various attributes of each test. At present there appear to be no protocols available for testing effects on microbial processes. In estimating the utility of each potential test, generality, nearness to validation, and simplicity were emphasized. Less emphasis was placed on sensitivity because this property is generally unknown for any broad range of chemicals. Similarly, suitable positive controls are generally lacking for all tests; their development will be the best approach to estimating the sensitivity of a test.

Test systems that directly or indirectly measure carbon and nitrogen mineralization were carefully evaluated because of their importance in most ecosystems. Direct carbon cycle tests include the evolution of $CO_2$ from soil with and without amendment of substrates. Direct nitrogen mineralization tests include the production of inorganic nitrogen from indigenous organic matter or amended nitrogeneous substrate. Most of the direct tests have been shown to be valid measures of ecosystem microbial processes. Rates of $CO_2$ evolution and nitrogen mineralization in laboratory tests are well correlated with field processes (e.g., Bunnel et al. 1977; Stanford and Smith 1972). We believe that responses to toxic chemicals could also be validated.

Oxygen uptake methods using Warburg techniques are commonly used to measure microbial activity in soil (for description of methodology, see Parkinson et al. 1971). As pointed out by Stotzky (1965), carbon dioxide evolution is more appropriate than oxygen uptake as a measure of microbial respiration in soil. Among the limitations of oxygen uptake methods are: (1) gases other than $CO_2$ may be evolved as a result of microbial activity and may interfere with the manometric measurement; (2) for oxygen uptake to be an accurate reflection of soil respiratory activity, the soil environment must be completely aerobic; (3) under anaerobic conditions, carbon dioxide is evolved without oxgyen uptake, and non-biological factors may interfere; and (4) chemicals acting as uncouplers of oxidative phosphorylation may produce erroneous results.

Problems also occur when $CO_2$ evolution measurements are used as indicators of microbial activity. These include the non-biological production of $CO_2$ through chemical decarboxylation, cell-free enzymes, or from free carbonates in soil. Phototrophic $CO_2$ fixation can be minimized by incubating samples in the dark, although in some soils chemoautotrophic $CO_2$ fixation may pose a problem.

TABLE 5.1. EVALUATION OF MAJOR TEST SYSTEMS FOR EVALUATING EFFECTS OF MICROBIAL PROCESSES[a]

| Assay test | Biogeochemical cycle | | | | Comments |
|---|---|---|---|---|---|
| | CHO | N | P | S | |
| I. Aerobic & General | | | | | |
| 1. $O_2$ consumption | X | | | | Too complex and difficult to measure |
| 2. Dehydrogenase assay | X | | | | Possible toxicity, interpretation problems |
| 3. Glucose $^{14}C$ mineralization | X | | | | Cost and apparent operator qualifications |
| 4. Enzymatic activities | | | | | |
| a. Protease | X | X | | | Possible lack of sensitivity |
| b. Cellulose | | X | | | Possible difficulty in establishing |
| c. Amylase | | X | | | Unknown validity |
| d. Phosphatase | | | X | | Unknown validity |
| e. Arylsulfatase | | | | X | Unknown validity |
| 5. Soil/substrate Additive - $CO_2$ | | | | | |
| a. Glucose | X | | | | Unknown validity/enrichment |
| b. Cellulose | X | | | | Unknown validity/enrichment |
| c. Starch | X | | | | Unknown validity/enrichment |
| d. Protein | X | | | | Unknown validity/enrichment |
| e. Plant material | X | X | | X | Probable validity |
| f. Soil humic material | X | X | | X | Poor sources commercially |
| 6. Heat evolution | X | X | | X | Too complex/costly |
| 7. Microbial biomass | X | | | | |
| a. Counts | X | | | | No meaning |
| b. Chlorophyll | X | | | | Surface soils only (no generality) |
| c. ATP | X | | | | Cost and apparent operator qualifications |
| 8. Nitrification | | X | | | Too sensitive/secondary microbial process |
| 9. Ammonification | | X | | | |
| a. Urea | | X | | | Poor generality/validity |
| b. Protein | | X | | | More validity |
| c. Plant material | | X | | | Most validity |
| 10. Nitrogen fixation | | X | | | Good validity |
| II. Anaerobic | | | | | |
| 1. Sulfate reduction with Desulfovibrio | | | | X | Minor biogeochemical importance |
| 2. Gas-generation ($CH_4$) | X | | | | Cumbersome and space consuming |

[a] Higher numbers indicate generally desirable attributes and lower numbers indicate disadvantageous attributes. High values for time, operator skill, and cost were taken to be advantageous (i.e., short-time, low skill or low cost).

TABLE 5.1 (continued)

| Assay test | Replicability | Standard-ization | Sensitivity | Generality | Equivo-cality | Statistical |
|---|---|---|---|---|---|---|
| I. Aerobic & General | | | | | | |
| 1. $O_2$ consumption | 2 | 1 | 3 | 3 | 2 | 0 |
| 2. Dehydrogenase assay | 2 | 3 | 0 | 2 | 1 | 0 |
| 3. Glucose $^{14}C$ mineralization | 4 | 3 | 3 | 3 | 3 | 0 |
| 4. Enzymatic activities | | | | | | |
| a. Protease | 1 | 2 | 0 | 1 | 2 | 0 |
| b. Cellulose | 1 | 2 | 0 | 1 | 2 | 0 |
| c. Amulase | 1 | 2 | 0 | 1 | 2 | 0 |
| d. Phosphatase | 1 | 2 | 0 | 1 | 2 | 0 |
| 3. Arylsulfatase | 1 | 2 | 0 | 1 | 2 | 0 |
| 5. Soil/substrate Additive - $CO_2$ | | | | | | |
| a. Glucose | 2 | 3 | 2 | 3 | 2 | 0 |
| b. Cellulose | 2 | 3 | 2 | 3 | 2 | 0 |
| c. Starch | 2 | 3 | 2 | 3 | 2 | 0 |
| d. Protein | 2 | 3 | 2 | 3 | 2 | 0 |
| e. Plant material | 2 | 2 | 1 | 3 | 2 | 0 |
| f. Soil humic material | 2 | 2 | 1 | 2 | 2 | 0 |
| 6. Heat evolution | 1 | 1 | 0 | 3 | 2 | 0 |
| 7. Microbial biomass | | | | | | |
| a. Counts | 1 | 1 | 1 | 1 | 1 | 0 |
| b. Chlorophyll | 2 | 2 | 0 | 0 | 1 | 0 |
| c. ATP | 2 | 1 | 0 | 2 | 1 | 0 |
| 8. Nitrification | 3 | 2 | 4 | 1 | 2 | 0 |
| 9. Ammonification | | | | | | |
| a. Urea | 3 | 3 | 2 | 1 | 2 | 0 |
| b. Protein | 3 | 3 | 2 | 2 | 2 | 0 |
| c. Plant material | 2 | 2 | 2 | 3 | 2 | 0 |
| 10. Nitrogen fixation | 4 | 3 | 3 | 2 | 2 | 0 |
| II. Anaerobic | | | | | | |
| 1. Sulfate reduction with Desulfovibrio | 3 | 3 | 0 | 1 | 2 | 0 |
| 2. Gas-generation ($CH_4$) | 1 | 0 | 0 | 3 | 2 | 0 |

TABLE 5.1 (continued)

| Assay test | Rejection standards | Failure frequency | Training expertise requirement | Time requirement | Cost per test | Positive controls |
|---|---|---|---|---|---|---|
| I. Aerobic & General | | | | | | |
| 1. O$^2$ consumption | 0 | 0 | 2 | 3 | 2 | 0 |
| 2. Dehydrogenase assay | 0 | 0 | 2 | 2 | 3 | 0 |
| 3. Glucose $^{14}$C mineralization | 0 | 0 | 1 | 3 | 1 | 0 |
| 4. Enzymatic activities | | | | | | |
| a. Protease | 0 | 0 | 2 | 2 | 2 | 0 |
| b. Cellulose | 0 | 0 | 2 | 2 | 2 | 0 |
| c. Amylase | 0 | 0 | 2 | 2 | 2 | 0 |
| d. Phosphatase | 0 | 0 | 2 | 3 | 2 | 0 |
| e. Arylsulfatase | 0 | 0 | 2 | 3 | 2 | 0 |
| 5. Soil/substrate additive - CO$_2$ | | | | | | |
| a. Glucose | 0 | 0 | 3 | 3 | 3 | 0 |
| b. Cellulose | 0 | 0 | 3 | 3 | 3 | 0 |
| c. Starch | 0 | 0 | 3 | 3 | 3 | 0 |
| d. Protein | 0 | 0 | 3 | 3 | 3 | 0 |
| e. Plant material | 0 | 0 | 3 | 2 | 3 | 0 |
| f. Soil humic material | 0 | 0 | 3 | 2 | 3 | 0 |
| 6. Heat evolution | 0 | 0 | 1 | 2 | 1 | 0 |
| 7. Microbial biomass | | | | | | |
| a. Counts | 0 | 0 | 2 | 1 | 1 | 0 |
| b. Chlorophyll | 0 | 0 | 2 | 3 | 3 | 0 |
| c. ATP | 0 | 0 | 1 | 2 | 1 | 0 |
| 8. Nitrification | 0 | 0 | 3 | 1 | 2 | 0 |
| 9. Ammonification | | | | | | |
| a. Urea | 0 | 0 | 3 | 2 | 2 | 0 |
| b. Protein | 0 | 0 | 3 | 2 | 2 | 0 |
| c. Plant material | | 0 | 3 | 2 | 2 | 0 |
| 10. Nitrogen fixation | 0 | 0 | 2 | 2 | 2 | 0 |
| II. Anaerobic | | | | | | |
| 1. Sulfate reduction with | 0 | 0 | 2 | 1 | 2 | 0 |
| 2. Gas-generation (CH$_4$) | 0 | 0 | 2 | 1 | 2 | 0 |

Indirect tests include various enzymatic assays, ATP assays, heat production, and estimations of microbial populations. Most of the indirect tests have not yet been shown to be generally valid measures of microbial processes or generalizable to different ecosystems. As pointed out by Parkinson et al. (1971), the use of the dehydrogenase assay has not been considered a useful quantitative method for assessing metabolic activity of microorganisms in soil because it is reputed to have less than 5% of the efficiency of oxygen uptake measurements. However, some workers have provided data that indicate this method can provide rough comparative estimates of microbial activity. Other tests based on enzymatic activity (e.g., protease, cellulase, amylase phosphatase, and arylsulfatase - see Table 5.1) have limitations because many extracellular enzymes can persist in soil in which microbial activity is inhibited; these tests are not based on growth related processes. Because of these limitations, we concluded that such tests would have dubious value as indicators of effects of chemicals in the natural environment.

Tests that include radioisotopic methods (e.g., glucose $^{14}C$ mineralization) are considered to have limited use because of cost and training requirements associated with use of a radioactive substrate. Likewise, the usefulness of tests based on heat evolution is limited because they are time-consuming and difficult to perform. Measurements based on microbial biomass (counts, chlorophyll, ATP) are difficult to perform and interpret.

To summarize discussions on currently available methods, it appears that the measurement of $CO_2$ evolution is the most practical and meaningful test for measuring the effects of chemicals on microorganisms involved in the carbon cycle.

(e) Proposed alternate tests. Group A proposed a test system to measure the effects of chemicals on carbon and nitrogen mineralizations simultaneously using environmentally relevant, high nitrogen substrates and mixed microbe populations that can be manipulated easily by technicians with minimal training. The test uses a soil system and can be modified for aerobic or anaerobic testing.

The basic test design involves the use of either a standardized protein (Pharmamedia or a similar product) or ground alfalfa meal as a substrate amendment and measurement of the following parameters at appropriate time intervals under aerobic or anaerobic conditions:

| Assay | Aerobic | Anaerobic |
|---|---|---|
| $CO_2$ evolution | * | * |
| $NH_4$ | * | * |
| $NO_2$ and $NO_3$ | * | |

For this set of assays the following matrix of treatments would be used:

| Test chemical | Substrate<br>- | <br>+ |
|---|---|---|
| - | a | b |
| + | c | d |

The characteristics of the proposed substrates are summarized in Table 5.2. Flasks or small specimen bottles, up to 500-800 mL capacity, were suggested for use in the assay. Approximately 30 to 40 g of wet soil could be used in each test flask. The soil should be at approximately 60 to 70% of water holding capacity in a layer not more than 10 mm in depth to assure that oxygen diffusion will not be limited in the nominally aerobic system. The jar should be set up to contain a small vial of alkali that can be exchanged at desired assay times if the same jar will be used for sequential assays. Sequential sampling of soil from the same flask should be avoided because it interferes with $CO_2$ measurement.

For anaerobic systems, the 30 to 40 g of soil per test sample could be placed in appropriate screw-cap top [illegible] x 70-mm test tubes with sufficient water to water-log the samp[illegible] assure that the systems will become anaerobic through [illegible] depletion processes. In addition, the soil-water [illegible] be purged with nitrogen to remove most of the dissolv[illegible]

TABLE 5.2. CHARACTERISTICS OF PROPOSED SUBSTRATES

| Advantages | Disadvantages |
|---|---|
| **Standardized Protein** | |
| Can be purchased to standard specifications - easily reproducible. | Responses are due to a limited part of the microbial population. |
| Easily soluble, and no subsequent direct mixing problems. | Microbial responses may be too rapid to allow usefulness in detecting differences due to the test chemicals. |
| Within limits, N and S levels can be specified. | |
| **Alfalfa** | |
| Plant materials are represented in an environmentally relevant form. | Insolubility may lead to inhomogeneous dispersion. |
| C, N, and S cycling can be monitored. | Grinding and mixing may influence rates of activity and make interpretation difficult. |
| | Reproducibility of materials from different sources may be difficult to assure. |

Any soil falling in the range of properties listed in Section A-3.5 (d)(i) (U.S. EPA, 1979 p. 162F) would be appropriate for the proposed microbial functions test. The soil should be either moistened or dried to 60 to 70% of the water holding capacity. Increments of the test substance should be added to the soil as water solutions and mixed thoroughly with the soil to yield the desired soil concentrations. If the test substance does not have adequate water solubility, it can be dissolved in an appropriate organic solvent that can then be added to the soil. Residual solvent would be evaporated. When this approach is used, all controls should be treated with equivalent volumes of the solvent alone. Soil water content should be checked after solvent evaporation and readjusted if necessary. Another strategy that can be used is to add the test chemical plus solvent to perhaps 10 to 25% of the test soil and, after solvent evaporation, to mix this treated soil with a larger volume of regular soil, using appropriate solvent controls.

As a screen, a single $CO_2$ assay can be performed at perhaps day 3 or 4 for the substrate-amended system, and days 7 to 11 for the plain soil systems. This will require a total of 24 flasks per test chemical (Table 5.3). For kinetic studies a schedule similar to that shown in Table 5.4 that allows the $CO_2$ assays to be completed with incubation in 12 days (2 work weeks) could be used. With this type of schedule, a series of sequential tests could be conducted biweekly. Per assay day, 12 or 24 assays would have to be completed. Inorganic N or other mineral nutrient determinations would be made on two soil samples at day 0 and on all test soil after the last $CO_2$ assay. Additional replicates would be required if a positive control is used.

TABLE 5.3. NUMBER OF $CO_2$ MEASUREMENTS PER TEST

| | Assay | | |
|---|---|---|---|
| | Sequential | | Single |
| | Unamended | Amended | |
| Assay times | 3 | 6 | 1 |
| Replications | 2 | 2 | 2 |
| Substrate amendment | 1 | 1 | 2 |
| Test substrate concentrations | 6 | 6 | 6 |
| | 36 | 72 | 24 |

TABLE 5.4. SCHEDULE FOR SEQUENTIAL SAMPLING: AEROBIC SYSTEM

| | Time | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | T | W | T | F | S | S | M | T | W | T | F |
| Day | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Substrate amended | Setup | x | x | x | x | | | x | | | | x |
| Substrate not amended | Setup | | | | x | | | x | | | | x |

Alternate tests proposed by Working Group A employ natural soil microflora, composed of many species, and substrate amendments that range from those that are easily decomposed (protein) to those that are more persistent (plant materials and soil organic matter). Major ecosystem processes that are monitored include carbon, nitrogen, and possibly sulfur mineralization and nitrification. Tests that are simpler than our test system include pure and mixed culture systems with similar or probably less complex substrates. Our proposed tests should confirm most negative results from simpler tests.

If a test chemical has no effect on $CO_2$ production from a variety of substrates by a pure culture, the same result will nearly always be obtained in a complex soil system. However, simpler systems may give positive results that are not confirmed by the proposed test system. In the simpler, pure culture systems, absorption and/or other physico-chemical fates of the test substances are minimal. However, in a more complex soil system, absorption of the test substance on organic matter or inorganic soil components may effectively lower the concentration of potential toxicants to levels that are no longer inhibitory. Futhermore, in multispecies systems, succession or adaptation to the potential toxicant may occur, resulting in little noticeable changes in the levels of C or N mineralization. However, it is possible that a microbial culture would partially degrade a test substance into a more toxic form. Because this is more likely to occur in a mixed culture, this system could be more sensitive than single species cultures in a few cases.

Tests that involve use of a ground plant material and soil with its natural microbial community, sacrifice some potential reproducibility for clearer validity in extrapolating test results to actual microbial processes. The use of positive controls in such tests would facilitate comparisons between different laboratories that use different soils or plant material.

(2) Protocol development. Development can be considered in terms of standardization and validation. Considerable work has been done on the laboratory measurement of microbial processes for agronomic and ecological investigations and to a lesser extent for studies of pesticides and other toxic substances. Despite this considerable experience, none of these systems have been standardized and shown to give comparable results among laboratories when used to test the effects of chemical substances. Such standardization must occur before any protocol is adopted. Validation studies are needed for all of the proposed tests. Various definitions of validation should be considered in development of these research strategies.

As noted in previous discussions and in Table 5.1, the use of protein or ground alfalfa decomposition in a soil environment appears to be well suited for use in evaluating the possible effects of test materials on terrestrial microbial processes. This is based on the ability to measure both carbon and nitrogen mineralization in the same system and to evaluate the release of carbon and nitrogen from natural substrates (protein and alfalfa) and from the native soil organic matter, processes that involve the functioning of varied groups of soil microorganisms.

In addition to this test, as noted in Table 5.1, several other assays are considered to be potentially useful. These include $^{14}C$ glucose mineralization, nitrification, and $CO_2$ evolution from a series of general substrates, including glucose and cellulose. The $^{14}C$ glucose mineralization assay, in spite of its known sensitivity, will probably not be useful because of its high cost, the needs for specialized equipment and radioactivity control requirements, and the needs for specialized training and clearances for technicians. Carbon dioxide evolution from glucose could be considered; this involves a soluble substrate, but a major concern with this substrate is that it would only allow evaluation of carbon processing performed by a narrow range of soil microorganisms. Cellulose, as noted earlier, is a difficult substrate because of its insolubility and difficulties in mixing it into test systems. In addition, pure cellulose is not a common material in soil systems.

Assuming that the test systems proposed by Working Group A might be considered for use in hazard evaluations, the following factors will require further development:

1. Optimization of soil-substrate and soil-test substances ratios.
2. Establishment of minimum amounts of C and N mineralization needed to complete assay.
3. Optimization of incubation time for control and substrate-amended systems.
4. Establishment of experimental variability and number of replicates required.
5. Use of known toxic agents to evaluate sensitivity of the assays.
6. Development and testing of a sampling schedule that will be useful under a wide range of conditions.
7. Selection of a test incubation vessel of minimum cost and simplifying the procedures of the test as much as possible.
8. Evaluation of the utility of sequential versus single assay analysis for the evaluation of $CO_2$ evolution, ammonification, and nitrification.
9. Selection of the best measurement procedure for evolved $CO_2$, ammonium, and combined nitrite and nitrate.

### 5.2.2 Identification of Model Ecosystems

(1) Test descriptions. Model ecosystems were divided into two categories, those that are synthesized in the laboratory and those that are excised from natural systems. A reference and a brief description of each system considered are provided below. The order in which they are listed does not represent a ranking.

(a) Synthetic systems.

Coleman and Anderson. A 50-mL Erlenmeyer flask with 20 g of seived sterilized soil and a defined (gnotobiotic) community including species of bacteria, protozoa, and nematodes (Anderson et al. 1978).

H. T. Odum. A 16-cm diameter desiccator with soil, litter, one transplanted bromeliad, fern, lichen, moss, and algal clump (Odum 1970).

Lichtenstein. A 86-mm diameter, 1-L plastic cylinder containing layers of treated and untreated soil and corn seedlings. Leachate is collected (Lichtenstein et al. 1977).

Lighthart and Bond. A plastic lined, No. 300 can or 600-mL beaker with 150-g homogenized soil and 15-g sifted litter (Lighthart and Bond 1976).

Metcalf and Cole. A 19-L, wide-mouth jar containing vermiculite, corn seedlings, earthworms, isopods, slugs, saltmarsh caterpillars, and a vole (Cole et al. 1976).

Nash and Beall. 150-cm-long, 115-cm-high, and 50-cm-wide, closed glass box with a 15-cm layer of seived soil and crop plants. Air and leachate are monitored (Nash et al. 1977).

Rosswall. A plastic pot with sterilized forest soil and litter, a defined soil biota, and a pine seedling (Rosswall et al. 1978).

TMC. The terrestrial microcosm chamber consists of a glass box (1 m x 0.75 m x 0.61 m) with 20 cm of synthetic soil, alfalfa, ryegrass, nematodes, earthworms, enchytraeid worms, isopods, mealworms, crickets, snails, and a pregnant vole. Air and leachate are monitored (Gile and Gillett 1979).

(b) Excised systems.

Grassland Core. A 15- or 30-cm diameter intact soil core with vegetation retained, encased in heat shrunk plastic (Jackson et al. 1979; Van Voris et al. 1978).

Outcrop. 90- x 90-cm sections excised from communities that develop in depressions in granite or sandstone outcrops arranged in 1- x 6.5-m concrete simulated outcrops (McCormick and Platt 1962).

Soil Core. A 5-cm-diameter by 5 to 10-cm-deep, intact soil core with vegetation clipped at ground level, encased in heat shrunk plastic (Jackson et al. 1977).

Treecosm. An approximately 2-m-tall sapling in 45 cm x 45 cm x 25 cm excised block of forest soil, sealed at the sides and contained in a plywood box (Jackson et al. 1978).

(2) Measurable parameters. Table 5.5 shows the community and ecosystem responses that have been or can be measured in each of these systems. Net primary productivity is the only one of these parameters that has direct social relevance. This parameter may be slow to respond, however, if it is mediated by changes in reproduction, soil fertility, or other intermediate factors.

A second category of parameters was considered relevant because of their relation to primary production and because they are likely to be worth measuring in model ecosystems. The first of these is loss of mineral nutrients (total N, $NO_3$-N, $NH_3$-N, Ca, P, K, S) and dissolved organic carbon. The utility of this parameter as an early indicator of ecosystem stress was hypothesized by O'Neill et al. (1977). It has been partially confirmed by a series of microcosm experiments conducted at Oak Ridge National Laboratory (ORNL) (Harris 1980) and Corvallis Environmental Research Laboratory (CERL) (Gile et al. 1979). A number of field studies indicate that nutrient dynamics are sensitive to both perturbations and internal successional processes (Jackson and Watson 1977; Likens et al. 1970; Richardson and Lund 1975; Best and Monk 1975; and Vitousek et al. 1979).

Respiration is another potentially important and readily measurable attribute of model ecosystems. The more common methods of measuring respiration in terms of $CO_2$ output have technical problems. Static absorption of $CO_2$ in KOH solution is inefficient unless respiration rates are low; static absorption on soda lime is more efficient but can cause drying of the soil. Use of a flow-through system can increase the efficiency of KOH absorption and eliminate the drying problem with soda lime, but both absorptive methods can have low precision because of errors in titration or weighing. Infrared gas analysis is precise and efficient, but has a high initial cost. The problems associated with measuring ecosystem respiration are discussed in more detail in the following references: Eckardt 1968; Monteith 1968; Woodwell and Botkin 1970; Odum 1970; Edwards and Sollins 1973; Minderman and Vulto 1973; and Van Voris et al. 1978.

The ratio of carbon to major mineral nutrients was also considered to be a potentially important indicator of system disruption. Rates of nitrification and the mineralization of nitrogen

## TABLE 5.5. PARAMETERS MEASURABLE IN TERRESTRIAL MODEL ECOSYSTEMS

| | Test Systems | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters and Attributes* | Metcalf + Cole | Lichtenstein | Nash | TMC | Coleman + Anderson | Rosswall | Lighthart | Odum | Soil core | Grassland core | Treecosms | McCormick et al. (granite outcrops) |
| Diversity | | | P | P | | | | | | Y | P | P |
| Succession | | | | | | | | | P | P | | |
| Trophic level interaction | | | | | | | | | | | | |
| Competition | | | P | Y | Y | P | | | | | | Y |
| Net primary productivity | Y | Y | Y | Y | Y | Y | | P | | Y | Y | Y |
| Nutrient retention | P | P | P | P | Y | Y | | P | Y | Y | Y | P |
| Respiration | Y | | P | P | P | | Y | Y | Y | Y | Y | Y |
| C/Nutrient ratio | P | P | P | P | Y | P | P | | P | P | P | P |

Y: The parameter has been measured in this system.

P: It is possible to measure the parameter in this system.

Blanks: The parameter cannot be measured in this system.

*Trophic level interactions cannot be measured in any of these systems.

and other mineral nutrients are tied to carbon dynamics (Cairns 1963; Johnson and Edwards 1979; and Johnson and Edwards 1980). Thus, simultaneous measurement of $CO_2$ production and mineral nutrients may supply a more sensitive and explanatory indicator of toxic response than either parameter alone. Mineral nutrients may be measured in leachate or in soil subsamples.

A third category of parameters consisted of those that could possibly be measured in model ecosystems but were not recommended because they present technical problems and are not necessary for the understanding of ecosystem response. ATP and enzyme assays are technically difficult, and the results are not easily interpreted. Community characteristics such as diversity and succession could only be measured with microbes or micro-invertebrates because of space and time limitations. These groups present severe taxonomic problems and the analysis would be difficult and time consuming. Interactions between specific species, such as competition and predation, are limited to the same groups of organisms and present the same problems unless simple gnotobiotic systems such as Coleman and Anderson's are used.

### 5.2.3 Evaluation of Model Ecosystems

(1) Synthetic systems.

Coleman and Anderson. This system is rated good for replicability, standardization, and cost of maintenance. It is rated low for level of training and frequency of failure because of the difficulty of maintaining gnotobiotic conditions. Its ability to represent real ecosystems is questionable. It may have potential for assessing effects on specific microorganismic couplings.

Lichtenstein. This system is rated very good for testing effects on primary productivity; it has good potential for testing effects of nutrient loss from soil. Its rating is good for replicability, standardization, cost, and level of training.

Lighthart. This system is rated good for replicability, standardization, level of training, and cost. It is rated low for applicability to testing ecosystem effects.

Metcalf and Cole. The utility of this system is limited in terms of substrate realism and validity of productivity measurements. It is not well designed for determination of effects on ecosystem processes. It is rated good for replicability, standardization, cost, and level of training required.

Nash. This system is good for measuring productivity. It has good replicability and standardization. It is rated low in terms of cost and size. It is not applicable in its current design for testing ecological effects.

H. T. Odum. This system is rated moderate for replicability. The level of training required is low, and it is low in cost. Its applicability to toxicants is good. It has a relatively high failure rate because of transplantation.

Rosswall. This system is rated good for replicability and standardization. It is limited in terms of small size and productivity. It is rated good in level of training required and cost.

TMC. This system is rated good for testing effects on competition and productivity, and potential for revealing effects on soil processes. It is limited by cost and size, and to some extent by level of training required. It is rated good for replicability and standardization.

In general, artificial soil substrates may increase replicability and standardization at the expense of application to testing effects on processes in real ecosystems.

Homogenized soil systems are most applicable to agricultural ecosystems and less applicable to uncultivated ecosystems. They are also more replicable and more easily standardized than intact microcosms.

(2) Excised systems. Grassland microcosms are considered more desirable than forest systems for testing purposes because of less restriction on size. However, size may be most limited by the need for expensive environmental control systems and labor costs in excision. Minimum sizes suggested were 10 to 20 cm in diameter and no less than 15 cm in depth.

In excised systems, replicability and standardization are difficult problems that need additional study before definitive recommendations can be made. In general, replicability should improve as size increases.

The measurements of ecosystem processes in these systems can be standardized, but the test systems themselves are defined by the ecosystems from which they are excised.

(3) Uncertainties concerning all model ecosystems.

Size. Despite some work on the effects of size on the responses of soil cores (Harris 1980), the effects of system size on most system types and parameters is unknown.

Soil Types. The physical and chemical properties of soils profoundly affect the fate and toxicity of chemicals. It is not clear that a sufficiently limited number of regionally representative soil types can be defined.

Boundary Effects. It is not clear whether the boundaries of a model ecosystem, which contrast with the continuity of natural ecosystems, affect the responses observed.

Construction. The container for the model ecosystem can affect system response by adsorption, channelization, and release of constituent chemicals into the soil.

Variance. The trade-offs involved in reducing variance by using larger systems or artificial substrates have not been defined.

Field Verification. While the utility of model ecosystems for studies of the fate of chemicals has been field validated, almost no work has been done to field-verify toxic responses observed in model ecosystems (Jackson et al. 1979).

Equivocality. The relationship of the parameters measured in model ecosystems such as $CO_2$ and nutrient output to socially relevant parameters such as primary production is not well defined.

### 5.2.4 Protocol Development for Model Ecosystems

None of the test systems are sufficiently developed to serve as test protocols. Nevertheless, the following test systems are worthy of further development:

Coleman and Anderson. These defined (gnotobiotic) systems are more likely to have explainable results than the undefined systems. Level of training and rate of failure resulting from gnotobiotic technology are impediments to their use in routine testing. These systems may have the potential for overcoming the disadvantages of the homogenized and excised systems as they are currently conceived.

Intermediate-sized grassland microcosms. Homogenized and excised microcosms of this type come close to meeting many of the criteria (size, cost, level of training, replicability, etc.) addressed at this workshop. However, further developments are needed in terms of round-robin analysis, field validation, and replicability. In addition, $CO_2$ analysis should be refined further. Large excised systems such as the excised tree microcosms and rare systems such as McCormick's outcrop microcosms may serve as excellent research tools for ecosystem processes. However, the size and expense of these systems may limit their usefulness for testing chemicals.

The small microcosms (5x5 cm) offer advantages in screening chemicals on the basis of the number of units that can be examined per unit cost. However, their inability to sustain primary production and the small volume of soil involved seriously limits the interpretation of results.

## 5.3 Conclusions

### 5.3.1 Microbial Processes

Any test for predicting terrestrial ecosystem effects on microorganisms should be as close to the natural system as reasonably possible. Certain pure culture systems could be developed that are more sensitive to toxic effects than the whole soil population. However, these pure culture systems ignore the complex interactions occuring in soil and the genetic variability and diverse nature of soil microbial populations. Therefore, it is extremely difficult, if not impossible to extrapolate the results of such pure culture systems to the natural situation.

Tests using natural soils were considered more meaningful than tests using soil suspensions that disturb the soil structure and substructure. Furthermore, soil slurry systems are very sensitive to oxygen transfer effects. Therefore, tests based on soil slurry systems may be difficult to standardize.

The natural system is not static but rather a dynamic system undergoing constant change. With the exception of nitrification, all microbial processes in the carbon, nitrogen, and sulfur cycles are performed by a diverse group of microorganisms. Because of this genetic diversity and the ability of microbial populations to adapt to the presence of a chemical, effects may be only temporary. Therefore, to mimic the natural system, kinetic (sequential) testing, rather than static testing, should be employed. This is not to say that a single measurement may not be useful as an initial screen for detecting possible inhibitory effects to microorganisms. However, to extrapolate the meaning of this single measurement to the environment, kinetic studies are required. Preferably, these kinetic studies would initially be performed in the laboratory and followed by field studies.

In addition to using natural soil microorganisms and the soil matrix as a test system, we concluded that measurement of effects of general metabolic processes occurring in the whole population or community (e.g., $CO_2$ formation, $O_2$ consumption) would be more meaningful than results from more selective tests based on a single enzymatic criterion (e.g., sulfatase, phosphotase, amylase). Because many of these extracellular enzymes can function in soil even after cell death, the results of these tests are difficult to interpret.

Considerable effort has been directed at studying the ability of microorganisms to transform chemicals in the terrestrial environment. In contrast, there are only a few studies defining the effects of chemicals on the microorganisms involved in the carbon, nitrogen, and sulfur cycles. Because of the genetic diversity of microorganisms, their ability to detoxify or polymerize certain pollutants, and the binding of pollutants to soil, it remains to be determined whether microorganisms in the terrestrial environment are sensitive indicators of the effects of chemicals. The development of suitable techniques for evaluating the effects of chemicals on microorganisms and their use to test compounds such as pesticides and polychlorinated biphenyls that have well established toxicity to other forms of life will lead to an answer to this question by providing a basis for comparison.

### 5.3.2 Model Ecosystems

No model ecosystem, synthetic or excised, is considered ready to serve as a test protocol. Nevertheless, defined systems such as Coleman and Anderson's and intermediate-sized grassland microcosms are recommended for further development. In addition, the following research activities are recommended:

1. Evaluation of encasement materials in terms of leachabiltiy, adsorptive capacity, optical properties, and durability.
2. Round-robin evaluation in all cases.
3. Field validation in all cases.
4. Determination of the relevance of measured parameters (nutrient loss) to major ecosystem processes.

### 5.3.3 General Discussion

During the general discussion that concluded the workshop, a number of common concerns were raised. One was the degree of realism required in a test system. Although we agreed that a minimum degree of realism is necessary, increasing realism involves greater cost and technical difficulty. The larger number of physical and biological elements in more realistic systems tends to reduce replicability. They also reduce sensitivity by increasing absorption and degradation of the test substance and by increasing the functional redundancy in the system. Finally, the presence of multiple biological elements can interfere with the interpretation of results in terms of the processes involved. For example, the presence of plants creates a more realistic environment for soil microbes, but plant respiration and nutrient uptake interfere with the measurement of mineralization and microbial respiration.

Therefore, it is necessary to determine whether a particular simplification of the test system will cause a qualitative difference in response. Brian Spalding suggested that the many years of experience in agronomic microbiology indicate that sieved soil adequately represents the dynamics of major nutrients in the field. However, the toxicant responses of whole natural ecosystems have not been well studied. Peter Van Voris suggested that the ecological importance of interactions between microbes, animals, plants, soil, and litter makes them worth considering in a testing scheme.

A second major issue is the effect of variation in the chemical, physical, and microbiological properties of soil on responses to chemicals. We agreed that, while this problem is difficult and highly significant, the direct approach of providing a standard test soil is impractical. The best alternative is to set limits on the major physical and chemical properties of test soils and then to use positive control substances to determine the relative sensitivity of each test soil. One must also recognize that variation in soil properties can affect the choice of response parameters. For example, nitrate loss was found to be a good indicator of toxicant response for soil cores from Oak Ridge but not for cores from Corvallis (Gile et al. 1979).

Finally, the problem of dose delivery and scheduling was briefly considered. Because TSCA controlled substances, unlike pesticides, are usually not deliberately released into the environment, the proper mode of delivery is not obvious. Test substances could be delivered in sprays, irrigation water, organic solvents, particulates, or vapors.

Substances could be delivered in a single acute dose or in continuous or episodic chronic doses. The mode of dose delivery can significantly affect the outcome of a test and, therefore, must be carefully considered in the development of a hazard assessment scheme.

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# METHODS FOR MEASURING EFFECTS OF CHEMICALS ON AQUATIC ECOSYSTEM PROPERTIES

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# SECTION 6

# METHODS FOR MEASURING EFFECTS OF CHEMICALS ON AQUATIC ECOSYSTEM PROPERTIES

## 6.1 Introduction

This workshop explored the potential uses of laboratory experimental ecosystems for assessing the effects of chemicals on aquatic ecosystem structure and function. The workshop objectives were:

1. To produce as complete a list as possible of laboratory experimental systems that have been (or could be) used to measure effects of chemicals on aquatic ecosystem properties.
2. To evaluate the capabilities of each model ecosystem for measuring system-level properties.
3. To evaluate each model ecosystem as a tool for routine screening of chemicals.
4. To outline logical test protocols for three different types of model ecosystems.

The workshop participants were divided into two working groups designated the Lentic Working Group (Group A) and the Lotic Working Group (Group B). The Lentic Working Group considered nonflowing freshwater model ecosystems and various marine systems; the Lotic Working Group discussed model stream research. Each working group addressed the following three general topics in a series of three working sessions:

Working Session I: Identification and Description of Test Systems

1. Identify model aquatic ecosystems (Group A: lentic; Group B: lotic) that display community and ecosystem-level properties. If possible, organize these model ecosystems into categories that are sufficiently well defined to permit generalization about their potential utility in hazard assessment requirements of the Toxic Substances Control Act (TSCA).
2. List community and ecosystem-level properties measurable in these model ecosystems.

3. Construct a matrix with test systems on the horizontal axis and measurable properties on the vertical axis. For each intersection in the matrix, assess the feasibility of measuring that property on that model ecosystem.

Working Session II: Evaluation of Test Systems

1. Construct a matrix with test systems on the horizontal axis and experimental criteria on the vertical axis. For each intersection in the matrix, evaluate that test system in terms of that criterion. The criteria are:

Replicability
Potential for interlaboratory transfer
Sensitivity to chemical stress
Generalizability to other aquatic ecosystems
Unequivocality of results
Statistical basis for interpreting results
Existence of standards for rejecting results (test failure)
Frequency of test failure
Level of training and expertise required
Time required
Cost per chemical
Others

Working Session III: Protocol Development

1. Write a first-approximation protocol for testing chemicals for effects on ecosystem properties. Of course, many questions would remain to be resolved before these protocols could be adopted by the Environmental Protection Agency (EPA)--these questions should be identified as they arise in the protocols. An outline for a protocol could be as follows:

I. Hypotheses to be tested by this protocol

II. System design

Size
Abiotic and biotic components
Light, temperature controls
Strategies for maximizing replicability

III. Test Procedure

Controls
Replicates per treatment; treatments per chemical

Introduction of test chemical into system
Measurement of effects

IV. Analysis of Results

Statistical basis for identifying effects
Criteria for rejection of test results
Interpretation of results
Generalization to other aquatic ecosystems

V. Development Needs

How to validate the test
Major questions that need to be resolved before finalizing protocol

## 6.2 Results and Discussion

### 6.2.1 Test Descriptions and Measurable Ecosystem-Level Properties

(1) Lentic Working Group. Seventeen model ecosystems or model ecosystem types within the scope of this workshop were listed (Table 6.1). Various approaches to organizing the list into general classes were suggested. Classification schemes based on distinctions between open and closed systems, between static and flow-through systems, between synthesized (gnotobiotic) and naturally-derived systems, between systems with and without sediments, and between large and small systems were all rejected because the variety of model ecosystems makes such categorizations unworkable. The list of 17 was later condensed to 9 representative systems [Section 6.2.2(1)].

A detailed list of measurable ecosystem properties was then compiled (Table 6.2). Because some of these properties were operationally defined, the list did not clearly distinguish between system-level parameters per se and methods that are commonly used for measurements. For the purposes of constructing an evaluation matrix, each property or method on the list was considered individually.

The model ecosystems in Table 6.1 were then evaluated in terms of their suitability for measuring the properties in Table 6.2. A simple 3-level rating system was used, with a rating of 1 indicating that a parameter was easy to measure in a given system; a rating of 3, difficult; and a rating of 2, intermediate. The full matrix is presented in Table 6.3.

In general, Group A thought that nearly all the ecosystem-level parameters could be measured in any of the model ecosystems, but that some measurements were more difficult than others. Destructive sampling over time is difficult in smaller systems; therefore, the number of measurements that can be made on a single experimental unit is limited. Sampling of larger, more complex systems is complicated

## TABLE 6.1. MODEL ECOSYSTEMS
(Lentic Working Group)

1. Gnotobiotic mixed flask culture (Nixon 1969; Taub 1969)

2 Derived mixed flask culture, closed (Corden et al. 1969)

3. Derived mixed flask culture, open (Leffler 1977; Neill 1975; Thomas 1978)

4. Carboy microcosm (McConnell 1962)

5. Pelagic community (Harte et al. 1978)

6. Pond community (Brockway et al. 1979; Giddings and Eddlemon 1979)

7. Artificial food chain, aquatic (Isensee et al. 1973)

8. Artificial food chain, terrestrial/aquatic (Metcalf et al. 1971)

9. Salt marsh box core (Kitchens 1979)

10. Marine pelagic, 150 L (Perez et al. 1977)

11. Marine littoral, benthic (Henderson et al. 1976)

12. Estuary, compartmentalized (Cooper and Copeland 1973)

13. Marine sediment core (Pritchard et al. 1979)

14. Freshwater sediment core (Medine et al. 1980)

15. Sediment-water systems, general (Neame and Goldman 1980)

16. Narragansett Bay, 13 $m^3$ (Pilson et al. 1980)

17. Marine plankton, deep tank (Strickland et al. 1969)

TABLE 6.2. MEASURABLE ECOSYSTEM PROPERTIES
(Lentic Working Group)

A. Chemical matrix (Eh, pH, etc.)

B. Electrical potential

C. Nutrient levels (mass balance)

D. Nutrient flux

E. Dissolved organic carbon

F. Biomass

G. Chlorophyll

H. Fluorescence

I. Turbidity

J. ATP

K. Size spectrum of particles

L. Taxonomic description

M. Primary production, respiration, P/R

N. Oxygen dynamics (input-output)

O. Heterotrophic activity

P. Dehydrogenase activity

Q. Enzyme systems

R. Grazing rate

S. Colonization rate

T. Spatial/temporal patterns

TABLE 6.3. EVALUATION OF MODEL ECOSYSTEMS FOR MEASURING ECOSYSTEM PROPERTIES[a]

| | | | | | | | Model Ecosystems | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ecosystem Properties | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. | 10. | 11. | 12. | 13. | 14. | 15. | 16. | 17. |
| A. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| B. | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| C. | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| D. | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 |
| E. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| F. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| G. | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 1 | 1 | 1 | 1 | 4 | 1 | 1 | 1 | 1 |
| H. | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 1 | 1 | 1 | 1 | 4 | 1 | 1 | 1 | 1 |
| I. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| J. | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 1 |
| K. | 1 | 1 | 1 | 1 | 1 | 3 | 4 | 4 | 1 | 1 | 1 | 1 | 4 | 3 | 3 | 1 | 1 |
| L. | 2 | 2 | 2 | 2 | 3 | 3 | 4 | 4 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 |
| M. | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| N. | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| O. | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| P. | 4 | 4 | 4 | 4 | 1 | 1 | 4 | 4 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 |
| Q. | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 1 | 3 | 3 | 3 |
| R. | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 |
| S. | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 2 | 4 | 4 | 1 | 4 | 4 | 4 |
| T. | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 |

Explanation of Ratings:
1 = Relatively easy to measure
2 = Intermediate difficulty
3 = Relatively difficult to measure
4 = Property not applicable or not measureable in this system

[a] See Tables 6.1 and 6.2 for identification of model ecosystems and properties, respectively.

by the difficulty of spatial heterogeneity within each system, which necessitates a stratified sampling regime.

Chemical and physical parameters are relatively easy to measure in the model ecosystems considered. Autotrophic activity is also easily determined (except, of course, in systems lacking autotrophic components). Heterotrophic activity is more difficult to measure because of the methods now available. Nutrient levels are easy to measure, but large numbers of samples may be required to overcome spatial and temporal variability. (Measurement of nutrient flux, as opposed to instantaneous concentrations, is more difficult; radioisotopes are generally needed). Finally, taxonomic descriptions are difficult and time consuming in all but the simplest model ecosystems.

(2) Lotic Working Group. Three major classes of lotic model ecosystems were recognized: (a) closed (completely recirculating) systems, (b) partially recirculating systems, and (c) open (once-through flow) systems. Each class was further broken down as shown in Table 6.4. These system types generally fall along a gradient from small, completely recirculating laboratory devices to large-scale, outdoor streams. Examples of each type are given in Table 6.4.

Measurable system-level parameters were identified and classified as structural or functional (Table 6.5). "Transport" in this list refers to all downstream (or upstream) movement of organisms and of dissolved and particulate matter, rather than toxicant transport in the usual sense; "fate" refers to the chemical fate of the toxicant. Except for transport, fate, and behavior, the properties listed by the Lotic Working Group correspond closely with those listed by the Lentic Working Group.

The Lotic Working Group used a three-level rating system, similar to that of the Lentic Working Group to assess the feasibility of measuring particular properties in each model ecosystem category. By and large, all properties are measurable in all systems. The major exception to this generalization is the closed recirculating tube system, which is too small and ecologically incomplete for some measurements. The matrix of model ecosystems vs. ecosystem properties is presented in Table 6.6.

### 6.2.2 Evaluation of Test Systems

(1) Lentic Working Group. Of the original list of 17 model ecosystems, nine were evaluated as TSCA testing protocols. This condensation was achieved by grouping similar systems into one category, and by including only those experimental systems that were considered to have the most potential. The condensed list forms the vertical axis of Table 6.7.

TABLE 6.4. MODEL ECOSYSTEMS
(Lotic Working Group)

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Closed systems

Closed recirculating tubs (e.g., Cushing and Rose 1970)
In-Situ (e.g., Bott et al. 1978)
Recirculating trough (e.g., Kevern and Ball 1965)
Laboratory channel (e.g., Kehde and Wilhm 1972)

Partially recirculating systems

Boxes, tubes, etc. (e.g., McIntire et al. 1964)
Laboratory channel (e.g., Brocksen et al. 1968)

Open systems

Wood or concrete channels (e.g., Maki and Johnson 1976)
Stream-side flume (e.g., Armitage 1980)
In-stream flume (e.g., Manuel and Marshall 1980)
Large-scale outdoor stream (e.g., Kania et al. 1976)

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### TABLE 6.5. MEASURABLE ECOSYSTEM PROPERTIES

(Lotic Working Group)

#### Functional

Primary production
Community production
Community respiration
Secondary production
Nutrient dynamics
Biochemical measurements
Functional group diversity
Transport
Behavior
Fate

#### Structural

Standing stock
Diversity - equitability
Colonization (recolonization)
Physical properties (pH, DO, DOC, etc.)

### TABLE 6.6. EVALUATION OF MODEL ECOSYSTEMS FOR MEASURING ECOSYSTEM PROPERTIES

(Lotic Working Group)

| Ecosystem properties | Closed systems | | | Partially recirculating | | | Open systems | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Functional: | Closed recirc. tubes | In-situ | Recirculation trough | Lab channel | Boxes, tubes, etc. | Lab channel | Wood and concrete channels | Stream-side flume | In-stream flume | Large-scale outdoor |
| 1° Production | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Comm. Production | N | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Comm. Respiration | M | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 2° Production | N | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Nutrient Dynamics | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Biochemical | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Functional Group Diversity | N | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Transport* | M | N | M | M | M | M | Y | Y | Y | Y |
| Behavioral | N | M | M | Y | Y | Y | Y | Y | Y | Y |
| Fate | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Structural: | | | | | | | | | | |
| Standing stock | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Diversity-equitability | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Coloniz., Recol. | N | N | N | N | M | M | Y | Y | Y | Y |
| Physical prop. (pH, DO, DOC, etc.) | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |

*Dissension expressed

Explanation of Ratings:

Y = YES; N = NO; M = MAYBE

## TABLE 6.7. EVALUATION OF TEST SYSTEMS

(Lentic Working Group)

| Model ecosystem type[a] | Replicability | Standardizability[b] | Training required (days) | Equilibrate?[c] | Acute effects (weeks) | Chronic effects (months) | Set up cost | Operation cost[d] | Sensitivity |
|---|---|---|---|---|---|---|---|---|---|
| (1) | G | G | PC[e] | - | 1 | 2 | + | - | ? |
| (2),(3) | G | G | 1 | + | 1 | 2 | - | - | ? |
| (5) | G | G | 1-7 | - | <1 | 2 | - | + | ? |
| (6) | G | G | 1-7 | + | 1 | >3 | - | - | ? |
| (7) | F | G | PC | - | 1 | 1 | + | - | ? |
| (9) | F | G | 1-14 | + | 4 | 2 | + | + | ? |
| (10),(11) | G | G | 1-14 | - | 4 | 2 | - | + | ? |
| (13) | G | G | 1-2 | - | ½ | 1 | - | - | ? |
| (14) | G | G | 1-21 | - | 2 | 1 | + | - | ? |

[a] Numbers refer to Table 6.1

[b] G = Good
F = Fair

[c] + = equilibration necessary
- = equilibration not necessary

[d] + = relatively expensive
- = relatively inexpensive

[e] PC = experience with pure cultures needed

Because time for discussion was limited, seven criteria were selected for evaluating these systems. These criteria are listed on the horizontal axis of Table 6.7. The following conclusions were reached:

1. Replicability. Most of the systems were judged to be fairly replicable, although replicability is essentially a function of the parameter being measured. Gross parameters, such as total primary productivity, are more similar between replicate microcosms than population-level measurements. It was also observed that variations in some properties over time may be out of phase between replicates; replication can still be considered good if replicate systems behave the same even though fluctuations are out of phase.

2. Interlaboratory transfer. All of the systems considered could be built and operated at different laboratories without difficulty.

3. Training required. All of the systems are fairly easy to set up and to operate. Estimates of the time needed to train an inexperienced technician to use these systems ranged from 1 day to 3 weeks. The synthetic systems require expertise in maintaining pure stock cultures. The measurement of ecosystem properties would be the most difficult aspect of these tests. The amount of training required would therefore depend on which parameters were being measured.

4. Time required. The time required to conduct tests with model ecosystems is a function of experimental objectives and measured responses. Some of the systems require an equilibration time after set up to allow conditions to stabilize before beginning an experiment. Acute effects might be measurable in as little as 6 h; chronic effects could be observed for 3 months or longer.

5. Cost of testing. None of the systems are particularly expensive to set up or operate. Taub's gnotobiotic systems are probably more expensive than most of the naturally-derived systems considered, because the gnotobiotic systems require maintenance of stock cultures. The salt marsh models of Kitchens and the 3-phase sediment cores of Medine and Porcella include some simple plumbing, which would increase their costs. For all of the tests, the major expenses are chemical analyses and other costs of measuring ecosystem responses. As with any chemical testing system, some expenses may be incurred in protecting workers from exposure to potentially harmful materials.

6. Sensitivity. The sensitivity of these systems was considered to be dependent on the response parameters measured rather than on the test systems themselves. It is not yet known which responses are most sensitive to chemical stress; this is an area where more basic research is needed.

7. Generalizability. Extrapolating from laboratory tests to natural systems was recognized as the major problem in model ecosystem research. To some extent, the generalizability of model ecosystem results depends on the responses being measured. Two basic strategies for making ecosystem-level tests "representative" emerged from the discussion. One approach is to construct a generic ecosystem (i.e., a system exhibiting important ecosystem properties but not mimicking any particular natural ecosystem). Such a system could be used to rank chemicals in order of toxicity, in the early stages of hazard assessment. A second approach is to model a selected natural ecosystem as closely as possible, so that microcosm results could be taken as indicative of probable effects in the natural ecosystems; the problem then becomes one of generalizing from one natural ecosystem to other natural ecosystems. A predictive, or mimicking, model ecosystem would probably be most useful in later stages of hazard assessment.

(2) Lotic Working Group. The 10 general categories of model ecosystems discussed in Working Session I were evaluated according to 13 criteria important to a hazard assessment process. The results of this evaluation are summarized in Table 6.8. The criteria were defined as follows:

1. Replicability. The Lotic Working Group interpreted "replicability" to mean ease of setting up replicate systems. Smaller systems are more replicable, by this definition, than larger systems. The question of similarity between replicates was not discussed.

2. Interlaboratory transfer. The methodology of smaller model systems is more easily transferred between laboratories than that for larger, more complex systems.

3. Sensitivity. Small systems are probably more sensitive to chemical stress than larger systems. However, sensitivity is primarily a function of the responses measured.

4. Generalizability. The ability to relate model ecosystem results to natural ecosystems was judged to be the most important criterion for evaluating model ecosystems as chemical testing tools. Small, closed systems were rated poor in this regard; only larger, open systems are enough like natural streams to permit reliable predictions. Even

TABLE 6.8. EVALUATION OF TEST SYSTEMS
(Lotic Working Group)

| Criteria | Closed systems | | Partially recirculating | | | Open systems | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Closed recirculating tubes | In-situ | Recirculating trough | Lab channel | Lab channel | Boxes, tubes, etc. | Wood and concrete channel | Stream-side flume | In-Stream flume | Large-scale outdoor |
| Replicability | G | F | G | G | G | F | F | F | P | P |
| Potential for interlab. transfer | G | G | G | G | G | F | P | P | P | P |
| Sensitivity to chemical stress | G | G | G | G | G | F | F | F | F | F |
| Generalizability to other aquatic ecosystems | P | P | P | F | F | F | F-G | F-G | G | G |
| Linearity | U | U | U | U | U | U | U | U | U | U |
| Unequivocality of results | G | G | G | G | G | G | G | G | G | G |
| Statistical basis for interpreting results | G | G | G | F | F | F | P | P | P | P |
| Existence of standards for rejecting results (test failure) | G | G | G | F | F | F | F | F | F | F |
| Frequency of test failure | U | U | U | U | U | U | U | U | U | U |
| Level of training and expertise required | L | L | L | M | M | M | H | H | H | H |
| Time required | L | L | L | M | M | M | H | H | H | H |
| Cost per chemical | L | L | L | M | M | M | H | H | H | H |
| Cause-effect interpretation | H | H | H | M | M | M | L | L | L | L |

H = High
M = Medium
L = Low

G = Good
F = Fair
P = Poor
U = Unknown

with larger model streams, doubts about ecological realism remain.

5. Linearity. This criterion refers to the effects of scale on the properties of model streams. To what extent can model ecosystem results be "scaled up" to full-sized natural streams? This question cannot be answered for any model ecosystem at present.

6. Unequivocality of Results. The group interpreted this criterion as "faith in the results". The conclusion was that any test system could be designed to provide unambiguous responses. However, the ability to interpret experimental results will depend on an improvement in our current knowledge of the complex interactions occurring in ecosystems.

7. Statistical Basis for Interpretation. Statistical analysis of results is easiest with small systems. The inherent variability of larger systems means that more samples are needed to achieve a given level of confidence in the measurements, and that temporal trends are more difficult to detect. The difficulty of replicating larger systems also detracts from the ability to detect statistically significant effects.

8. Standards for rejection. Simple standards for rejecting the results of any particular test might be possible for smaller model systems but less likely for larger ones.

9. Frequency of test failure. The frequency of test failure is unknown for any model stream, mainly because failures are rarely reported in the literature.

10. Training required, time required, and cost. Small, recirculating systems require less training, less time, and less money to operate than larger systems. The large, open systems received the worst ratings by these criteria.

11. Cause-effect interpretation. The complexity of a large, open system makes interpretation of chemical effects difficult. Direct toxic effects are not easily untangled from the web of secondary effects and interactions occurring in a complex system. Cause and effect are most easily distinguished in small, simple systems.

An interesting generality that emerged from this evaluation exercise was that systems meeting the operational criteria for a screening test (replicability, potential for interlaboratory transfer, statistical interpretation, low cost, short time, and low level of expertise required) are the same systems that are least generalizable to natural ecosystems. This echoes the conclusion of the Lentic Working Group that two types of ecosystem test systems are desirable--one for screening, the other for prediction. Rapid, simple screening tests cannot be designed without sacrificing ecological realism. Model ecosystems designed with ecological realism as their objective are most appropriate at later stages in the assessment process.

### 6.2.3 Protocol Development

The protocol development exercise served two functions. First, outlines of three possible ecosystem-level effects tests based on three widely-used model ecosystem types were produced. Second, the protocols constituted focal points for discussions of common problems encountered in system-level testing, and strategies for dealing with them.

There were interesting differences in the approaches taken by the working groups. The Lentic Working Group chose to develop two protocols, one involving sediment communities (freshwater or marine), and one including primarily pelagic organisms. The sediment protocol, adapted from methods of Medine (freshwater) and Pritchard (marine), provides for small-scale, simplified simulation of specific aquatic environments. The pelagic protocol is essentially the mixed flask culture method of Beyers, Leffler, and others; the experimental unit is a highly-simplified, naturally-derived community that simulates no specific natural ecosystem, but exhibits system-level properties common to all ecosystems. Both of these protocols were developed by selecting familiar model ecosystem designs that seemed most amenable to routine toxicity screening. The Lotic Working Group approached the task differently. They began by selecting a small number of system-level responses that were considered most ecologically meaningful, and then designed a model ecosystem that would reflect these responses as realistically as possible. The protocol that resulted from these deliberations is a nonrecirculating laboratory channel that, according to the conclusions reached in Working Session II, does not meet the requirements for a screening test but could be used in the advanced stages of a chemical hazard assessment. The Lotic Working Group thought that model streams simple enough to be used for screening would not represent natural streams realistically.

Each protocol obviously contains many unresolved problems, and none of the protocols could be used in hazard assessment without extensive refinement and validation. They are sketches of feasible ecosystem-level tests as envisioned by a group of experienced scientists. The protocols are presented here as prototypes for further research and development.

(1) Sediment core effects test procedure. This procedure tests the hypothesis that a chemical will alter the system-level properties (chemical matrix, primary production, heterotrophic activity, nutrient

cycling) of a sediment-water system. The test consists of cylinders containing homogenized sediment or intact cores, water, and natural biota; continuous or semicontinuous flow. Hypolimnetic, littoral, or marine benthic environments are simulated.

Cores are contained in 1- to 10-L cylinders, with depth 5 to 6 times the diameter. Cylinders may be lucite or glass; lucite is recommended for testing metals, but is unusable with some organic materials; glass is more expensive, but deteriorates less rapidly than plastic. A sediment core or homogenized sediment (depth equal to cylinder diameter) is placed in each cylinder. Cylinders are then filled with natural water (prefiltering not recommended) or defined medium (preferable for mass balance calculations). Biota are included with the sediment and/or water. Medium exchange is continuous or semicontinuous, with a residence time of 2 to 10 d. Cylinders are illuminated from above by horizontal Duro-Test lights (approximating sunlight spectrum) on a 12-h photoperiod (hypolimnion can be simulated by running test in darkness). Temperature is maintained at 15 to 25°C, depending on environment; temperature is controlled within 0.5°C by water jackets or by an environmental chamber. The water is stirred with sufficient mixing energy to prevent stratification without resuspending sediment. Aeration is not required.

A test should include two replicates per treatment, plus two controls (plus two carrier controls if appropriate). Each chemical is tested at three concentrations at least. The chemicals are introduced with the medium, or added separately by syringe pump; introduction of chemicals without a carrier is preferred, if possible. Homogenized cores should be equilibrated for 15 to 20 d before introducing the chemical; no equilibration is necessary with intact cores. Measurements to be made on treated and control systems include:

1. Chemical matrix (Eh, pH, TOC, DOC, contaminant levels).
2. Primary production and respiration (by light/dark bottle technique, measurement of diurnal dissolved oxygen fluctuations, or oxygen mass balance).
3. Nutrient levels, especially $NO_3$-N, $NH_4$-N, organic N.
4. Heterotrophic activity ($^{14}CO_2$ production from labelled compounds, algal lysate, or detritus; or carbon balance).

Measurements should be made at least weekly. Two weeks of exposure are required to determine no-effect levels; three sampling intervals are necessary to confirm the magnitude of effects observed.

Treated microcosms are compared with controls to identify effects of chemical treatment. Test results are rejected if controls or treatment are lost; controls should exhibit ecosystem measurements consistent with local environmental conditions. Interpretation of results is arbitrary until comparisons with other microcosm experiments or field studies can be made (high level of funding recommended for this purpose).

(2) Pelagic ecosystem effects test procedure. This procedure tests the hypothesis that a chemical will alter the system-level properties (chemical matrix, primary production, heterotrophic activity, nutrient cycling) of a generalized aquatic ecosystem. The test consists of bacteria, algae, and microinvertebrates maintained in artificial growth medium. Typical ecosystem properties are exhibited but no particular natural ecosystem is simulated.

Mixed aquatic communities are maintained in loosely-capped 4-L beakers. Samples of biota from local ecosystems are used to establish laboratory stock cultures; the taxonomic composition of these cultures is not important as long as major functional groups (autotrophs, grazers, detritivores, and decomposers) are present. When stock cultures reach a fairly stable taxonomic composition, they are used to inoculate the test communities. A standard growth medium (e.g., Taub's #63 or Gerloff's) is used. Cultures are stirred continuously or only during sampling. Light is supplied for 12 h each day. A constant temperature of 20 to 25°C is maintained. During the pretreatment period, replicates are cross-seeded periodically to increase uniformity of composition. Evaporative losses are replaced with equal volumes of stock culture.

Each test includes five controls plus five replicates per treatment. Chemicals are tested at three treatment levels corresponding to 1/10 X, 1 X, and 10 X the predicted environmental concentrations. After a 6-week equilibrium period, microcosms are treated once with the test chemicals; treatments are not repeated. Measurements on treated and control systems include:

1. Chemical matrix (Eh, pH)
2. Primary production and respiration (3-point oxygen method)
3. Nutrient levels ($NO_3$-N, $NH_4$-N, ortho-P, SRP, total C, DOC)
4. Heterotrophic activity ($^{14}CO_2$ release from labelled substrate, epifluorescent bacterial counts, or oxygen changes)
5. Autotrophic biomass (chlorophyll, phaeophytin, fluorescence)
6. Turbidity

Measurements that can be automated should be made twice daily (just before the lights turn on and just before they turn off); other parameters should be measured weekly. The systems should show a response within 2 weeks after the chemical is added. System response should be monitored for a period sufficient to allow recovery, or a maximum of 10 weeks.

Deviations of measured parameters from the 95% confidence limits of the normal operating range of controls are integrated over time to provide an index of effect. This index is "total relative stability" (Leffler 1977); it provides a single measure of an ecosystem's total stress response. This index can be compared with those produced by standard chemicals tested in the same systems. Any deviation from controls signifies a positive response, because 95% confidence limits are built into the analysis. Test results are rejected if the test system fails to rank four standard chemicals properly, or if the behavior of the controls is aberrant. The ranking of four standard chemicals serves as the criterion for assessing the reproducibility of the test system. Thus, even though the species composition and proportions may vary within each batch of microcosms, the batches are considered as the same test system as long as they rank the four chemicals in standard order.

All aspects of the protocol need much more testing and development. Methods of introducing chemicals must be refined. The optimal time frame of the test has not been established.

(3) Model stream effects test procedure. This procedure tests the hypothesis that a chemical will alter the system-level properties (primary production, respiration, community production) of a stream ecosystem. The test consists of laboratory-scale, nonrecirculating model streams, containing natural substrate and naturally derived biota. Regional characteristics of small stream ecosystems are simulated.

Model streams are assembled in indoor troughs, 2- to 10-m long and 30-cm wide. A substrate (limestone, gravel, rock) is placed in the troughs, and well water is pumped through the systems with a current speed of 0.01 to 0.04 m/sec. A naturally derived autotrophic community (system-dependent) and a nondrifting invertebrate grazer (cosmopolitan) are the major biota. Overhead lighting is provided at about half the normal sunlight intensity for the region. Temperature, pH, and DO are controlled within the normal regional range.

A single test includes two replicates at each of three treatment levels, plus two replicate controls. The effects of the chemical are measured after 2 to 4 weeks of continuous exposure. Primary production and respiration are measured on stream substrate communities placed in respiration chambers. Net community production is measured by destructive sampling and total biomass determination after 30 d exposure. Nonparametric procedures are used to compare treated streams with controls.

The protocol must be checked in interlaboratory round-robin tests. The protocol may be validated by comparing test results with conventional toxicity tests and any available field data.

## 6.3 Conclusions

Most ecosystem properties (primary productivity, secondary productivity, ecosystem respiration, nutrient cycling, total biomass, functional or structural diversity, etc.) can be measured in most model ecosystems. The ease with which a particular property may be measured, and the degree to which that measurement is representative of natural ecosystems, depends on the laboratory system and the property being measured. Research is needed to identify which properties are most easily measured, most sensitive to chemical stress, most critical to the functioning of the ecosystem, and most generalizable to other systems. This research must precede the adoption of standard testing protocols.

The model ecosystems that are the most replicable, inexpensive, simple, rapid, and easily standardizable are also the least realistic and most difficult to extrapolate to natural ecosystems. There seem to be two potential roles for model ecosystems in hazard assessment. The first role is a general, nonrepresentational model (i.e., mixed flask culture). This type of system exhibits universal ecosystem properties without mimicking any particular natural ecosystem. Such a system is easily replicated, simple, and cost efficient, and could be used early in the testing sequence to screen chemicals for their ability to disrupt ecosystem processes. The second role involves more detailed representation of specific natural ecosystems such as ponds, lakes, streams, or coastal environments. These systems can provide information on the magnitude and direction of ecosystem effects, as well as details about sensitive organisms, sensitive processes, indirect effects, and ecosystem recovery. Because of the greater expense and expertise required to use such model ecosystems and because their results are not necessarily generalizable to other ecosystem types, these systems are best used in the later stages of hazard assessment.

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# METHODS FOR MEASURING EFFECTS OF CHEMICALS ON TERRESTRIAL POPULATION INTERACTIONS

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# SECTION 7

# METHODS FOR MEASURING EFFECTS OF CHEMICALS ON TERRESTRIAL POPULATION INTERACTIONS

## 7.1 Introduction

This workshop considered the availability and utility of multispecies laboratory systems to display the responses of terrestrial population interactions to toxic chemicals. The workshop was divided into three working groups. Working Group A discussed interactions between microbe populations and within microbial communities. Working Group B discussed interactions between plant populations and between plants and microbes. Working Group C discussed interactions between arthropod populations and between arthropods and plants. Each working group participated in three sessions. The objectives of each session were outlined as follows:

Working Session 1: Identification and Description of Test Systems

Identify laboratory systems that display terrestrial population interactions or community properties. If possible, organize these systems into categories that are sufficiently well defined to permit generalization about their potential utility in the Environmental Protection Agency's (EPA) Toxic Substances Control Act (TSCA) hazard assessment processes.

List population and community properties measurable in each system.

Working Session II: Evaluation of Test Systems

Construct a matrix with test systems on the horizontal axis and experimental criteria on the vertical axis. For each intersection in the matrix, evaluate that test system in terms of that criterion. The criteria are:

Replicability

Potential for interlaboratory transfer

Sensitivity to chemical stress

Generalizability to other terrestrial ecosystems

Statistical basis for interpreting results

Existence of standards for rejecting results (test failure)

Frequency of test failure

Level of training and expertise required

Time required

Cost per chemical

Others

Working Session III: Protocol Development

Based on our experience to date, it may be possible to write a first-approximation protocol for testing chemicals for effects on certain population interactions. Of course, many questions would remain to be resolved before these protocols could be adopted by the EPA--these questions should be identified as they arise in the protocols. An outline for a protocol could be as follows:

I. Hypotheses to be tested by this protocol

II. System design

  - Size
  - Abiotic and biotic components
  - Light, temperature controls
  - Strategies for maximizing replicability

III. Test Procedure

  - Controls
  - Replicas per treatment; treatments per chemical
  - Introduction of test chemical into the system
  - Measurement of effects

IV. Analysis of Results

  - Statistical basis for identifying effects
  - Criteria for rejection of test results
  - Interpretation of results
  - Generalization of other terrestrial ecosystems

V. Developmental Needs

  - How to validate tests
  - Major questions that need to be resolved before finalizing protocol

Many other systems may seem appropriate for development. Identify the systems that seem to be most appropriate and describe the types of development that are necessary for each (e.g., testing of species, development of media).

What types of modeling or analysis would be necessary to relate test results to the real world, to generalize the results to a variety of environments, or to relate the parameters measured to socially significant parameters?

What simpler tests could be confirmed by the results of this multispecies test? What more complex test might confirm the results of this multispecies test?

## 7.2 Results and Discussion - Microbial Populations

The microbial process working group in the terrestrial ecosystem properties workshop (Section 5) considered the soil microbiota in terms of their contribution to ecosystem processes, that is, as a black box that changes the chemical characteristics of its substrate. In this workshop, Working Group A considered the internal structure of the black box at two levels. The first level consists of the basic interactions between pairs of populations: predation, parasitism, competition, antagonism, and mutualism. The second level is the community characteristics that are immediate products of population interactions: taxonomic composition, diversity, succession, and resistance to invasion. Each of these areas is discussed in the following sections.

### 7.2.1 Population Interactions

The feedback mechanisms associated with population interactions tend to moderate the effects of changes in the soil environment resulting in a weak homeostasis. Disruption of these interactions can be experimentally examined. The potential utility of these experimental systems for testing the effects of chemicals is evaluated in Table 7.1.

(1) Predation and parasitism. Predation and parasitism of bacteria can only be studied with reasonable ease in liquid cultures. Addition of a clay suspension to the cultures may provide an approximation of conditions in the soil. An example of such a culture system is the one devised by Roper and Marshall (1978). This system could be used with *Escherichia coli* as the host and *Bdellovibrio* as the parasite or with a protozoan as the predator. The system should be run in the dark at 25°C for 12 d. The response parameter is the concentration of host and predator or parasite cells measured over time with and without the test chemical. The purity and viability of the cultures must be monitored.

While the procedures for this test system are well established, the applicability of the test to real world conditions is questionable. The importance of these processes in the soil and the generality of responses measured in this system need to be determined. Field validation of this system would be difficult.

TABLE 7.1. RATING AND EVALUATION OF TEST SYSTEMS FOR MICROBIAL POPULATION INTERACTIONS

| Test | Priority[a] | Replicability | Potential for interlaboratory transfer | Sensitivity | Generality | Statistics | Frequency of failure | Training required (degree) | Time (days) | Cost (dollars) |
|---|---|---|---|---|---|---|---|---|---|---|
| Predation and parasitism | 4 | M | M | U | U | ANOVA | M | BS | 30 | 400 |
| Competition[b] | 5 | | | | | | | | | H |
| Lichens | 1 | H | H | H | H | ANOVA | L | BS | 7 | 500 |
| Methanogens | 2 | H | H | H | M | ANOVA | H | BS | 14 | 200 |
| Antagonism | 1 | H | H | U | U | Indices | L | BS | 42 | 200 |
| Survival | 2 | M | H | U | U | Regression and $ED_{50}$ | L | BS | 28 | 200 |
| Fungistasis | 3 | U | H | U | H | ANOVA | L | BS | 7 | 200 |
| Population levels | 5 | M | M | U | U | ANOVA | - | BS | 30 | 1000 |
| Succession | 5 | M | H | U | U | --- | - | BS | >60 | H |

H: High
M: Moderate
L: Low
U: Unknown

[a] A rating of expected relative utility on a scale from 1 (high) to 5 (low).

[b] No system specified.

(2) Competition. While microbial competition undoubtedly occurs in the soil, it is very difficult to measure. We recommend that competition be tested in flow-through systems with defined limiting nutrients that model aquatic systems more appropriately than terrestrial systems.

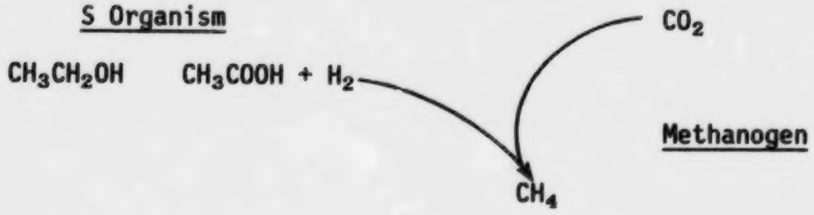
(3) Mutualism. Some microbial species enter into stable mutualistic relationships that perform ecological functions that cannot be performed by the individual symbionts. Toxicants can disrupt these mutualistic relationships.

(a) Lichens. The algal-fungal symbiosis that constitutes lichens has been shown to be sensitive to air pollutants and to physical factors that cause imbalance in growth rates of constituent species. Lichens are important components of tundra ecosystems and appear to contribute significantly to nitrogen dynamics in coniferous forests of the Pacific Northwest.

The primary response parameters are respiration, measured as either $O_2$ consumption or $CO_2$ output, photosynthesis, which may be measured as $^{14}CO_2$ assimilation, and the ratio of photosynthesis to respiration (P/R). In addition, $N_2$ fixation can be measured by acetylene reduction in lichens that contain $N_2$-fixing blue-green algae. Test chemicals may be applied as gases, mists, or soaking solutions. Care must be taken to ensure that the experimental conditions are appropriate, that is, that the control thalli remain healthy.

Lichens are particularly useful for measuring the effects of chemicals transported in the atmosphere. The P/R ratio may be a good indicator of responses that lead to overgrowth of one symbiont by the other. The mode of response of lichens to toxicants is poorly understood and their sensitivity has only been demonstrated for sulfur oxides and gaseous oxidants.

(b) Methanogenesis. Methane production can occur in chronically wet soils, rice paddies, swamps, and marshes. The "methanogenic consortium" performs interspecies $H_2$ transfer between heterotrophic, anaerobic bacteria and $H_2$-consuming methanogenic bacterial symbionts. Methanobacterium strain M.O.H. and the so-called S organism are used in the test system described by Bryant et al. (1967). This mixed culture converts ethanol to methane according to the following reaction.



The S organism is inhibited by its own product, $H_2$. But the $H_2$ is needed by the methanogen, which converts it to $CH_4$ and thus keeps $H_2$ levels low enough for the S organism to continue converting ethanol to $H_2$ and acetate. A chemical may disrupt this system by affecting either the S organism or the methanogen.

The test could use the procedure of Miller and Wolin (1974) with the two membered culture growing on ethanol, under strictly anaerobic conditions. The test chemicals should be dissolved in a prereduced mineral salts solution and introduced into the culture. Methane production should be measured using the syringe displacement method (Healy and Young 1979) or gas chromatography. Exposure to any $O_2$ will completely stop methanogenesis. Thus, rates of zero are suspect and should be repeated.

The test only applies to the few terrestrial ecosystems where $CH_4$ is naturally produced, such as landfills, swamps, bogs, rice paddies, and flooded soils.

(4) Antagonism. Some plant diseases are precluded by antagonistic relationships between microorganisms normally associated with the plant and pathogenic populations. For example, Rhizoctonia solani is a common plant pathogen having a wide host range. The presence of Trichoderma spp. in soil associated with the host induces suppressiveness to R. solani, effectively controlling the disease. Similar suppressiveness may be induced in certain soils by the presence of fluorescent Pseudomonas which are antagonistic to the Fusarium (vascular) wilt pathogens. Pseudomonas spp. are also important in plant growth responses. We hypothesize that the addition of a test chemical can alter the host-pathogen-microbial interactions characteristic of these systems.

Soils have become suppressive to plant pathogens when crops are grown in monoculture. A model system that demonstrates this phenomenon consists of repeatedly planting radishes at weekly intervals in soil infested with R. solani (Henis et al. 1978; Rouse and Baker 1978; Henis et al. 1979; Wijetunga and Baker 1979). In a typical experiment containing an initially low inoculum density, 100% disease incidence occurs after three replants. By the fifth replant, however, disease incidence has decreased to almost zero. Associated with this is the increase in propagule density of Trichoderma spp. from an initial density of $10^2$ propagules/g to $10^6$ propagules/g at the end of 5 weeks. The effects of a toxic substance on these interactions can be examined.

To maximize replicability, certain factors of environment and inoculum are important. The most important environmental factor influencing the system is soil pH. Trichoderma is most active in acid soils. Thus, the soil should have a natural or adjusted pH of around 6. Effects of the added chemical on soil pH would have to be taken into consideration. The other important factor is the size of the R. solani inoculum. When propagules (sclerotial) of size > 250 µm are used as inoculum in alkaline soils, suppressiveness is not induced, and there is no influence on propagule density of Trichoderma. However, suppressiveness is readily induced in acid soils when propagules of size > 250 µm are used as inoculum. If propagules of size < 250 µm are used, suppressiveness is induced and Trichoderma spp. increase in either alkaline or acid soils.

(a) Test procedure. Ten containers, each with 100 g of soil at a matric potential of 0.7 bar, are each seeded with 32 radish seeds. These are arranged in a radiating pattern with the aid of a vacuum seed planter. In five of the containers, inoculum of R. solani is introduced into the center. Five others are left as noninoculated controls. After a 1-week incubation period, a conducive index (CI) can be determined (described later). The inoculum is grown in a chopped potato-soil mix and is composed of both large and small propagules. If acid soil is available, this mixture can be used. If alkaline soil is used, the large propagules (> 250 µm) should be screened out. Containers are covered with clear plastic during incubation to maintain a relatively constant matric potential.

The chemical to be tested can be mixed into the soil at different concentrations at the time that the initial matric potential is established. One week after the first seeding and after the CI is determined, the plants are uprooted. All replicates of each treatment are combined and the soil redistributed (as before) in the containers. Thirty-two radish seeds are planted again in each container. This process is repeated at weekly intervals. Usually the soil develops suppressiveness in about 5 weeks (replants). At this time, fresh inoculum can be introduced into the center of the pot and the CI can be determined again.

During the period of the test, ambient laboratory temperatures are satisfactory for incubation. Light is supplied but need not be of high intensity because radishes are not grown for more than a week.

Development of suppressiveness in the soil during the course of the test is associated with increase in propagule density of Trichoderma spp. Some soils in nature contain low numbers of this microorganism ($10^2$ propagules/g in Fort Collins clay loam). One naturally suppressive soil from Bogata, Columbia, contains 8 x $10^5$ propagules/g. There are some soils that do not contain Trichoderma spp., and, in these, conidia of this fungus should be introduced at the beginning of the test.

(b) Analysis of results. Response parameters include CI and disease incidence (DI) for radishes grown in monoculture, the inoculum density of R. solani, and the propagule density of Trichoderma spp. during the course of the test.

CI is determined by the following equation:

$$CI = \frac{A - B}{A},$$

where A is the number of healthy plants in the noninoculated control, and B is the number of healthy plants in the inoculated treatment. The limits of the index are 0 to 1:0. If the CI is 0, the soil is completely suppressive, and if the CI is 1.0, it is completely conducive. DI is computed similarly:

$$DI = \frac{A - B}{A}$$

The difference in CI and DI lies in the experimental design. The CI is computed when inoculum is introduced into the center of the container and measures not only inoculum potential but also the ability of the pathogen to grow in soil. The DI is measured when inoculum is distributed randomly in soil after mixing and measures largely inoculum potential. There is no rationale for transformation of raw data accumulated to propagule densities of *R. solani* and *Trichoderma* spp. A graphic display over time is sufficient.

In most instances, differences (treated below) are so dramatic that statistics need not be applied to confirm differences. However, analysis of variance can be used if needed.

1. *Criteria for rejection of test results*. When the test is done properly and essential parameters adequately controlled, results in nontreated controls are predictable. The initial CI is usually 0.85± in a conducive soil. DI increases in inoculated treatments and is near 1.0 after the third replanting. By the fifth replanting, DI is quite low and may be near 0.1. The inoculum density of *R. solani* rises until the third replanting, diminishing subsequently to undetectable levels by the fifth week if small propagules are used initially. *Trichoderma* increases from barely detectable levels to near $10^6$ propagules/g soil in the 5-week period in inoculated treatments. If these phenomena do not develop in the controls, the validity of the test is questionable.

2. *Interpretation of results*. The test chemical may change these interactions by modifying the CI or DI, which would indicate changes in the antagonistic interaction of *R. solani* and *Trichoderma* spp. Changes in these interactions can be precisely monitored and, thus, readily detected.

3. *Generalization to other terrestrial systems*. Currently, in the research area of biological control of plant pathogens

in soil, interest is being centered on two groups of biocontrol agents that apparently contribute substantially to the level of suppressiveness found in soils. Species of Trichoderma comprise one of these groups. These fungi are microparasites and are representative of this type of antagonistic relationship among microorganisms found in soil.

(c) Development needs. The test may be validated in the field to see if a chemical has the impact observed in the laboratory. There appear to be no major technical questions requiring resolution before using the process.

### 7.2.2 Community Properties

There are two types of community properties. The first consists of properties, such as fungistasis and survival, that represent the action of the community as a unit on an indicator species. The second type consists of properties such as diversity, succession, and relative population levels that indicate the structural state of the community.

(1) Survival test. After a period of microbial activity in soil, environmental conditions become less conducive for continued growth and microorganisms produce propagules. The length of time that these propagules are capable of surviving is a function of numerous biotic and abiotic factors. The hypothesis of this test is that a chemical may influence survival directly, by acting on the introduced microorganisms, or indirectly by acting on the soil community.

Small containers, each containing 100 g (or less) of nonsterile soil, can be infested with the test organisms. These are incubated under standardized conditions of moderate temperature and moisture. No light is required.

Controls with and without the introduced organism should ensure that propagule density of the introduced microorganism is being monitored and not the contaminants. Five replicates should be sufficient.

The elements that may be found in a typical survival curve are diagrammed in Figure 7.1.

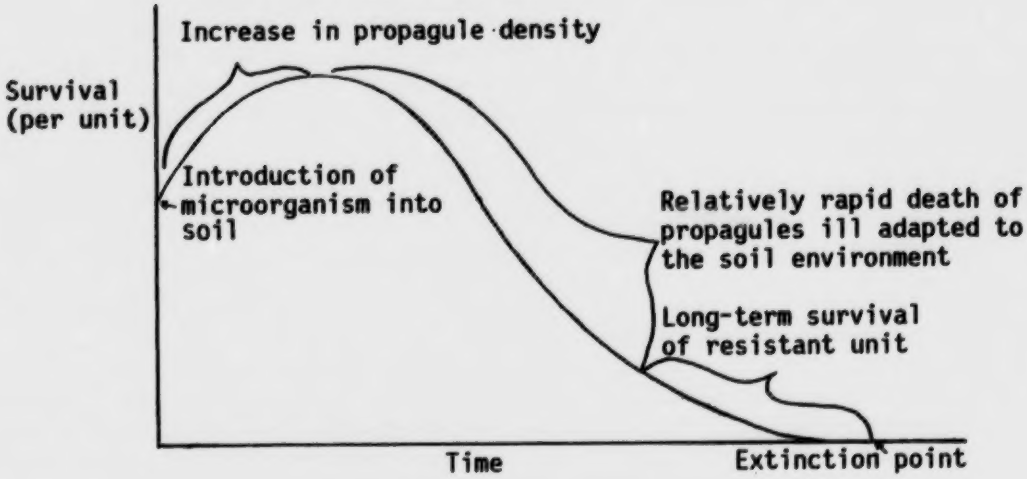


Figure 7.1. Typical survival curve.

When a microbial population is introduced into soil, propagule density may increase because of the availability of substrates in the soil. Some microorganisms may be introduced with the substrate on which growth took place, for example, dead tomato stems containing microsclerotia of Verticillium dahlea. In such cases, decay of the substrate releases the propagule units so that there is an apparent increase in density when the individual units are counted in assays.

After this period, there appears to be a relatively rapid increase in death rate because of the different capacities for survival among the propagules. A proportion of the units may persist for relatively long periods and are relatively resistant to the insults inflicted by the soil environment.

Because these resistant propagules represent long-term survival and are likely to survive in soils in nature, they should be assayed for persistance. Thus, after the system has reached equilibrium, the test chemicals should be introduced into the soil at various concentrations.

Test microorganisms should be selected that are typical members of soil microbial communities. Also, techniques should be available for determining propagule densities as aliquots of soil are assayed over time. We suggest bacteria such as Rhizobium and Agrobacterium tumefaciens as likely candidates because their cell densities in soil can be followed quantitatively with the amino fluorescence technique. Rapid assays, selective for Rhizoctonia solani (a fungus), are also available. In this case, the persistence of distinct propagule types (large and small) can be followed. The small propagules would be particularly susceptible to insults.

If the chemical reduces survival, it would be detected as an increase in death rate over time in comparison with a nontreated control. The extinction point need not be measured because interpolations and extrapolations may be obtained when the data are transformed by methods described later. Although the time required for the test would depend on the test organism, this obviously shortens the time to a matter of weeks rather than months or years.

Various transformations have been suggested. The semilog transformation assumes that propagules die at a logarithmic rate:

$$\ln y = y_0 + rt$$

The symbol y equals the number of viable units at a given time (t), and $y_0$ equals the number of viable units at 0 time. The rate (r) is negative because it describes the rate of death.

It may also be assumed that susceptibility of propagules to death follows normal distribution in time. Thus, the log-probit transformation may also be a legitimate candidate for mathematical descriptions of survival. In practice, the proportion of surviving propagules in probit units is plotted against time on a logarithmic scale.

Research has yet to be done to establish which of these transformations is better for data analysis. One study indicated that they were complementary. In an analysis of data obtained from various examples in the literature, the log-probit transformation appeared to give a better performance; that is, it seemed to give $TS_{50}$ (time required for 50% of the propagules to die) values and $TS_{10}$ points much closer to those observed in nature.

Slopes of transformed curves may be subject to regression analyses to obtain slope (r) values for comparisons. Test microorganisms can be selected that are representative of the microbial community.

(2) Fungistasis. Fungistasis is the failure of viable fungal spores to germinate in soil. Because the fungistatic activity of soil is removed by sterilization of the soil, anti-fungal activity is believed to be related to the activities of other microorganisms in the soil and is probably related to the ability of the soil microflora to withstand invasion of alien species. Several mechanisms have been proposed, but a satisfactory explanation of fungistasis has yet to be accepted. This test system is designed to determine if potentially toxic chemicals will alter the interrelationship between fungal propagules and the rest of the soil microbial community.

The test should be run in the dark at room temperature. The test chemical is added to the fungistatic soil, which is then placed in a Petri dish. A suspension of spores from species such as *Fusarium* solani or Aspergillus flavus (nutrient dependent) is passed through a 0.20-μm pore size filter so that the spores can be retained on the filter surface. Final spore density should be ~2000 per $mm^2$. The filter is then buried in the soil, and the Petri dish is covered. Plates are incubated at room temperature ~14 d (a time series may be run), and the percent of fungal spores that have germinated is determined microscopically after staining the filter with Lactophenol cotton blue. At least 1000 total spores per filter should be counted.

The following series of control tests should be run to elucidate the nature of the test chemical's effects:

| | |
|---|---|
| Steam sterilized soil | Fungal spores should germinate. Potential toxicant may act at this level and prevent spore germination. |
| Chemically sterilized soil | Plus 10 to 100 μl of ethanol/g of soil. All spores should germinate. (Other mixtures that may be more reliable, such as amino acid-carbohydrates, should be tested.) |
| Non-sterile soil | Concentration of the test chemical equals zero. No, or few, spores should germinate. |

Soil not fungistatic, as evidenced by a high degree of spore germination in non-sterile soil in the absence of the test substance, would cause rejection of the test. If fungal spores germinate in the presence of the test substance, then the natural fungistatic properties of the soil have been adversely affected by the chemical. Because fungistasis in a soil is strongly correlated with the activities of the indigenous microflora, the test substance has interfered with the interactions between these microflora and the invading fungal spores. Fungistasis has been found in all natural soils that have been examined. The degree of fungistasis is limited, however, in soils high in organic matter such as peats and mucks. Neither deep subsoils nor very acidic soils are fungistatic.

Laboratory development, interlaboratory testing, and field validation are necessary. To our knowledge the effect of any toxicant on fungistasis has yet to be assessed in this manner. The system needs development to determine adequate spore density per filter, length of incubation, soil moisture levels, etc.

(3) Population levels. Relative population levels of microbial species and taxonomic or functional groups could be determined as a measure of interference of toxicants with normal population balance mechanisms. Because of the difficulty of enumerating species, the following ratios are probably most useful.

1. Fungus/bacteria ratio. Enumerate bacteria and fungi by direct counting with acridine orange epifluorescence counting. This does not indicate viability.

2. Viable fungal propagule/bacteria ratio. Enumerate fungi and bacteria by viable plate count methods. Use Saboraud Medium for fungi; Trypticase soy agar for bacteria.

3. R/S ratio. Enumerate bacteria by direct count or viable bacteria (plate count) in rhizosphere (R) and in root-free soil (S).

Measure these ratios in soils and perform ANOVA to determine if addition of toxicant significantly alters the ratio. It is not possible to state the ecological significance of these ratios, but they are generally believed to be important.

(4) Succession. Succession is an important process that can be measured only at great cost. Significance of deviations of successional processes often would be difficult or impossible to evaluate. An exception would occur with preemptive colonization by microorganisms, such as *Lactobacillus*, that prevent further successional events. Preemptive colonization would be easily detected by monitoring other processes such as respiration. Therefore, it does not appear reasonable to examine successional events for microbial populations in soils or on leaf litter. Some early successional stages can be examined in flow-through systems and should be considered for aquatic ecosystem testing.

(5) Diversity. Diversity is a community-level parameter that may be used to measure stress in microbial communities. Stressed communities often have low diversities that can be quantitatively assessed by a variety of diversity indices such as the Shannon Index. Almost any substance can cause a shift in diversity in the microbial community. Normally, the diversity rapidly returns to its original level following a minor environmental insult. Exposure to a persistent toxic substance may result in a prolonged depression in microbial diversity.

Diversity can be assessed using the techniques of numerical taxonomy (Kaneko et al. 1977) or perhaps by direct microscopic observation (Staley et al. 1980). If numerical taxonomy is used, numerous isolates will be needed. Diversity may be used for monitoring the long-term major impact of a toxic substance. Measured changes in diversity can potentially be applied to all microbial communities, but the consistency of the response between communities and its validity in the field are unproven.

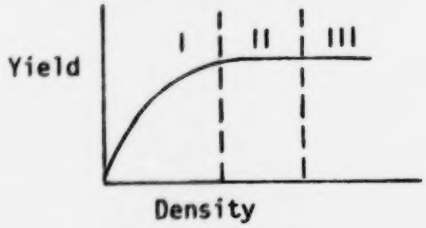
## 7.3 Results and Discussion - Plant and Microbe Populations

Working Group B considered systems that display interactions between species of vascular plants and between vascular plants and microbes. These classes of interactions are closely related because of the dependence of the competitive ability of a plant on its relation to microbial symbionts. Because there are no existing test systems for these classes of interactions, synopses of potential test systems are presented that are derived from the research experience of the working group participants. Each proposed system was evaluated and rated by consensus of Working Group B. The results are presented in Table 7.2.

### 7.3.1 Interference

Interference between plant populations includes competition for space, light, water, and mineral nutrients as well as allelopathy and other modifications of the medium. The best understood method for examining this process in the laboratory is to sow seeds of two plant species into pots. Two designs are possible. In the additive design, the density of one species is kept constant while different densities of a second species are added resulting in a variable total density. In the replacement series, the total density is kept constant while the proportions of the two species are varied.

Before a pair of species can be used, preliminary studies must be performed to determine appropriate pot size, nutrient levels, and total densities. Density selection is based on the following model.



In Phase I, little or no interference occurs and yield is simply the product of density and age-specific plant weight. In Phase II, yield is constant, but individual plant weight declines because of interference. This is the optimum density range for the test system. In Phase III, yield is constant, but mortality occurs. Controls should include each species grown alone. To permit allelopathic interactions, a sandy loam soil should be used, and watering should not leach the pots.

The following parameters should be considered:

1. Number of individuals for each species.

TABLE 7.2. RATING AND EVALUATION OF TEST SYSTEMS FOR PLANT-MICROBE AND PLANT-PLANT INTERACTIONS

| Test | Priority | Replicability | Interlaboratory transfer | Sensitivity | Generality | Statistics | Frequency of failure | Level of training required | Time (days) | Cost per chemical (dollars) |
|---|---|---|---|---|---|---|---|---|---|---|
| Interference (clover-fescue) | 1 | H | H | H | M[a] | ANOVA-GLM | L[b] | BS | 56 | 7000 |
| Agricultural microcosm | 3 | M-L | M | U | H | ANOVA | U | BS | 28 | 1000 |
| Endomycorrhizae -grass | 2 | M | M | H | H[c] | ANOVA | M | MS[d] | 84 | 3500 |
| Ectomycorrhizae -conifer | 2[e] | M | M[e] | H | H[c] | ANOVA | M | MS[d] | 14 | 2000 |
| Rhizobium-legume | 2 | H | H | H | M | ANOVA | L | BS | 21 | 200 |
| Wheat rust | 2 | H | H | M | L | ANOVA | L | BS | 21 | 1000 |
| Crown gall | 2 | H | H | H | L | ANOVA | L | BS | 21 | 100 |
| Rootknot nematode | 2 | H | H | M | M | --- | L | BS | 42 | 600 |

H: High
M: Moderate
L: Low
U: Unknown

[a] A very common pasture type but application to other systems is doubtful.

[b] Assumes good greenhouse practices.

[c] Mycorrhizae are not highly species specific.

[d] A high level of training is required to establish and maintain inoculum cultures.

[e] The rating would be 1 if commercial inoculum becomes available and the potential for interlaboratory transfer would be high.

2. Shoot and root dry weight; shoot weight alone is frequently adequate.

3. Nitrogen concentration of tissues.

4. Nitrogen fixation rate of legumes.

5. Concentration of other nutrient ions (P, K, Ca, Fe, Mg).

6. Seed production - if time allows.

From these data, species ratios, mean weight per plant, and mean weight per pot should be calculated.

The following pairs of plant species are potentially useful in this type of system:

1. Unstable perennial systems

   *Trifolium subterraneum* - *Lotium* sp.
   *Trifolium repens* cv Tillman - *Festuca arundinacea* cv Kentucky 31;

2. Early successional systems

   *Helianthus annus* - *Digitaria sanguinalis*
   *Sorghum halepense* - *Bromus* sp.
   *Erigeron canadensis* - *Aster pilosus*
   *Ambrosia* sp. - *Amaranthus retroflexus*;

3. Agricultural systems

   *Zea mays* - *Sorghum halepense*
   *Avena sativa* - *Hordeum sativum*
   *Glycine max* - *Panicum* sp.
   *Helianthus annuus* cv Russian mammoth - *Amaranthus retroflexus*;

4. Successional systems (later stages)

   *Andropogon virginicus* - *Pinus taeda* (seedlings inoculated with mycorrhizae).

Seed germination is good for these species, except for *Amaranthus*, which needs a temperature greater than 25°C; *Ambrosia*, which needs a cold period; and *Sorghum halepense* (Abdul-Wahab and Rice 1967). It helps to remove "seed coats" for consistent germination of *Helianthus*.

Experiments with these species can be completed within 8 to 10 weeks. Information regarding the above listed combinations are available in Harper (1977) and Rice (1974 and 1977).

The clover-fescue pair was considered the best single candidate. Experimental procedures have been developed, and results using pollutant gases as stressors have been published. A synopsis of the proposed procedures for this test is presented below:

> Conduct the test in a greenhouse with 2- to 2.5-L capacity plastic pots and a sandy loam-quartz sand mixture at a 3:2 ratio by volume. The temperature range should be 23° to 27°C. Water as needed (use tensiometer check), but water all plants short of saturation. Fertilize with VPHF - 5-15-10, a commercially available, water-soluble fertilizer with micronutrients, or equivalent, at a rate of 15 g/3.8 L $H_2O$ in a split application - 100 mL/pot at seeding and 100 mL/pot 10 d after germination. After germination thin to eight plants per pot.
>
> Avoid hot summer months. Use supplemental lights (mercury and sodium vapor) during a 14- to 16-h period to provide 70% full sunlight; analyze the growing medium prior to the test (cation exchange capacity, pH, mechanical analysis, organic matter, ion content, etc.); be sure that the N level is not so high as to suppress N-fixation by Rhizobium (this also keeps fescue in check); add Rhizobium inoculum prior to the test to standardize legume performance; 4 weeks after germination, add the test compound, then harvest the tops of the plants 2 weeks later; and determine dry weight of the total biomass for each species.

### 7.3.2 Mycorrhizae-Plant Interactions

Test systems are proposed for both of the major classes of mycorrhizae-endomycorrhizae and ectomycorrhizae.

(1) Endomycorrhizae-grass. This test incorporates effects on formation of the endomycorrhizal association as well as direct effects on cereal crops. A synopsis of the proposed test procedure is presented below:

> Several candidate grasses could be used: sorghum (2 to 3 plants per pot), millet (2 to 3 plants per pot), and corn (1 plant per pot). This test would be run in the greenhouse with pots, growing medium, temperature range, lights and water similar to those specified for the plant interference test. Fertilization could also be the same except that phosphorus levels must be maintained below 45 ppm. A multiple species mixture of Glomus spp. in the form of root fragments (infected) and adhering soil containing fungus spores can be used as inoculum (Kormanik et al.

> 1977). Add the root-soil inoculum to growing medium (1:10 by volume) prior to filling pots.
>
> Ten days after germination of the plants, add the test chemical. Twelve weeks after seeding, terminate the test, remove the roots and rinse them carefully; remove 2 to 4 random root samples (5 g/sample fresh wt.) from the roots from each pot. Clear and stain the roots by the method described in Kormanik et al. (in press). By microscopic examination, determine the frequency and degree of vesicular/arbuscular colonization in the selected feeder root segments.

(2) Ectomycorrhizae-conifer. This system is well studied and mycorrhizal inoculum may soon be commercially available. A synopsis of the proposed procedure for this test is presented below.

> Employ standard tree containers (125 cc capacity each cavity), loblolly pine seed, and peat-virmiculite growing medium (1:1 by volume). Prepare a vegetation inoculum of *Pisolithus tinctorius* dried to a bulk density of 350 g/L and mix to a ratio of 1:15 in the medium. Water as needed. Fertilize at 3 to 4 week intervals (NPK = 200:20:40). Greenhouse temperature should be 24 to 27°C. Supplement light as needed to give a 14- to 16-h day. Use an uninoculated control. Add the test chemical at seeding. After 1 or 2 weeks, remove 5 to 10 seedlings per tray and visually examine the roots for percent ectomycorrhizal development.

A growing medium for ectomycorrhizae is described in Marx (1969), inoculation procedures are described in Marx and Bryan (1975), and container production of inoculated seedlings is described in Ruehle and Marx (1977).

### 7.3.3 Rhizobium-Legume Interaction

While the clover-fescue interference test provides a test of the rhizobium-legume interaction, a test may be desired that does not include a second plant species to complicate interpretation of results or that simulates a legume row crop. The proposed procedure for this test is presented below.

> Start with a very low nitrogen soil medium. *Rhizobium* inoculum should be added to soil prior to potting. Watering, growth medium, pot size, light, and greenhouse temperature can be the same as in the interference test. Seed four beans (Bush-Blue Lake 290) and thin to one per pot. The test chemical should be added at planting. The test should be run with and without N fertilization. Run the test for 21 d. Measure the above-ground biomass production, describe visible signs of injury to the plant, and visually assess nodulation on root systems using broad categories: < 5%, 45%, 70%.

### 7.3.4 Wheat-Wheat Rust

This system includes a plant (*Triticum* spp.) and fungal pathogen (*Puccinia graminis* var. *tritici*) that are of major economic importance. The procedures, developed by the USDA Cooperative Rust Laboratory at the University of Minnesota in St. Paul, should be used for culturing, inoculation, and grading the reaction of the wheat to the rust (Stakman et al. 1962). The combination of wheat and rust varieties should be selected so that control plants give a moderately susceptible response to the rust fungus. The chemical should be applied at planting. Changes in the rust reaction (size of uredia) should be recorded 10 d after inoculation.

### 7.3.5 Carrot-Crown Gall

This system provides a simple and compact demonstration of bacterial plant pathogenesis. The NASA procedure (Wells and Baker 1969; Kleinschuster et al. 1975) for crown gall is recommended.

> Cut carrot disks and place them on moistened filter paper in a Petri dish. Place the bacterial inoculum and the diluted test chemical on the carrot disk. After 21 d determine the fresh weight of the galls.

### 7.3.6 Plant-Nematode Interactions

Nematodes are important components of the soil biota, and the rootknot nematode is a significant agricultural pest. This test system would utilize the procedure from the screening for resistance test developed at North Carolina State University (Taylor and Sasser 1978). The test chemical and nematode egg masses would be added to the soil at the same time the tomatoes are transplanted. After 5 or 6 weeks, giant cell development, extent of galling and nematode egg production would be determined. Tomato varieties that have been identified as moderately resistant (Sasser and Kirby 1979) should be used.

### 7.3.7 Agricultural Soil Microcosm

This system represents competition between agricultural and weed species. The following procedure is recommended for this test.

> Collect soil from a field that has been fallow for at least a year to avoid extremes of fertility and concentrations of agricultural chemicals. Soil pH should be in the range 5.5 to 6.0. Screen the soil, and mix with seeds of clover, horseweed, crabgrass, and fescue. Fill wooden flats approximately 40 cm x 15 cm x 6 cm deep; add the test chemical; place the flats in a greenhouse; water as needed but do not fertilize. Measure rate of emergence, survival, and biomass at termination (4 weeks).

## 7.4 Results and Discussion - Arthropod Interactions

Working Group C developed a set of "Type Arthropod Interactions" that were considered to have potential for evaluating the impact of toxic substances on terrestrial population interactions. The interactions were categorized as follows:

1. Arthropod interactions with plants as phytophagous feeders

    Sucking feeders
    Grazing (chewing) feeders

2. Arthropod interactions with biotic mortality factors (exploiters)

    Parasitoid
    Predator
    Pathogen

3. Interspecific competition

4. Symbiotic interactions

    Interspecific symbiosis
    Intracellular symbiosis

5. Functional interactions between sucking, grazing arthropod on a single plant unit

6. Host plant competitive interactions as mediated by a phytophagous insect.

The best studied sets of species for each of these interactions were identified and listed. From these lists, species sets were selected that are either proposed for development (Sect. 7.4.1) or that show some promise but cannot be recommended at this time (Sect. 7.4.2). Finally, a tentative test protocol is presented for competition between Tribolium species. Each of the interactions listed above is included in at least one of these systems.

### 7.4.1 Proposed Test Systems

The systems described below were designated as having the greatest potential for evaluating the effects of chemicals on arthropod population interactions. These systems use relatively well-studied species and can include more than one of the type interactions. The systems are evaluated in Table 7.3, and the constituent interactions are ranked in Table 7.4.

TABLE 7.3. RANKING AND EVALUATION OF THE PROPOSED TEST SYSTEMS

| System | Rank | Replicability | Interlaboratory transfer | Sensitivity | Statistics | Frequency of failure | Training[a] (degree) | Time (days) |
|---|---|---|---|---|---|---|---|---|
| Whitefly | 1 | | | | | | | |
| Whitefly-bean | | H | H | H | T-Test | U | BS[a] | 42-56 |
| Whitefly-Encarsia | | H | H | U | T-Test | U | MS | 42-56 |
| Corn earworm | 2 | | | | | | | |
| Earworm-corn | | H | H | L | T-Test | U | H.S.[b] | 80-90 |
| Earworm-Trichogramma | | H | H | M | T-Test | U | B.S./M.S. | 30 |
| Earworm-NP virus | | H | H | U | T-Test | L | B.S. | 14 |
| Earworm-nematode-bacterium | | M | M | H | --- | U | --- | 20-30 |
| Tribolium | 3 | H | H | H | Presence-Absence | L | H.S. | 180-365 |
| Housefly | 4 | | | | | | | |
| Housefly-parasitoid | | H | H | M | T-Test | U | H.S. | 60-180 |
| Housefly-blowfly | | L | H | M | T-Test | U | B.S. | <180 |
| Housefly-blowfly-parasitoid | | L | H | U | --- | U | B.S. | <180 |
| Alfalfa-aphid-parasitoids | 5 | H | H | H | T-Test | L | B.S. | 30-42 |
| Brown soft scale | 6 | | | | | | | |
| Plant-scale | | H | H | H | | L | B.S./M.S. | 28-56 |
| Scale-scale | | M | H | M | | L | B.S./M.S. | 28-56 |
| Scale-parasitoid | | H | H | H | | L | B.S./M.S. | 28-56 |
| Scale-predator | | H | H | M | | L | B.S./M.S. | 28-56 |

H: High
M: Moderate
L: Low
U: Unknown

[a] All of these systems require specialized training beyond the education level indicated.

[b] High School.

TABLE 7.4. RATING OF ARTHROPOD POPULATION INTERACTIONS[a]

| Interaction | Generality | Level of training | Time | Cost | Documentation | Sensitivity | Economic importance |
|---|---|---|---|---|---|---|---|
| Plant-herbivore | | | | | | | |
| Whitefly | 2 | 3 | 2 | 3 | 3 | 1 | 3 |
| Earworm | 1 | 1 | 4 | 1 | 1 | 4 | 1 |
| Aphid | 1 | 2 | 1 | 2 | 2 | 1 | 2 |
| Scale | 2 | 4 | 3 | 4 | 4 | 3 | 4 |
| Herbivore-exploiter | | | | | | | |
| Whitefly-parasitoid | 3 | 2 | 2 | 1 | 2 | 1/1[b] | 3 |
| Earworm-nematode | 2 | 3 | 3 | 3 | 1 | 4/1 | 1 |
| Earworm-parasitoid | 2 | 2 | 3 | 3 | 1 | 4/1 | 1 |
| Earworm-NP virus | 2 | 1 | 3 | 3 | 1 | 4/3 | 1 |
| Aphid-parasitoid | 2 | 1 | 1 | 1 | 2 | 1/1 | 2 |
| Scale-parasitoid | 3 | 1 | 2 | 1 | 3 | 3/2 | 4 |
| Scale-predator | 3 | 2 | 2 | 1 | 3 | 3/2 | 4 |
| Housefly-parasitoid | 1 | 1 | 2 | 1 | 2 | 4/1 | 3 |
| Competition | | | | | | | |
| Aphids | 1 | 1 | 1 | 1 | 2 | 2 | 1 |
| Scales | 3 | 2 | 2 | 1 | 4 | 2 | 3 |
| Housefly-blowfly | 2 | 1 | 1 | 1 | 3 | 3 | 2 |
| Tribolium | 4 | 1 | 2 | 1 | 1 | 1 | 1 |

[a] Ratings of 1 are favorable--high generality and sensitivity, low cost.

[b] Fraction indicates the sensitivity of herbivore/exploiter.

(1) Plant-whitefly-parasitoid. The greenhouse whitefly is a sucking herbivore that can use tomatoes, cotton, and a variety of other domestic plants, but beans are recommended for laboratory testing. It is exploited by the parasitoid Encarsia formosans, which is available commercially. Both insects have 21-d life cycles at 20°C. The system is documented in Burnett (1967).

(2) Corn-earworm-exploiters. The corn earworm, a lepidopteran, chewing herbivore, is an important pest of corn. Earworm exploiters that can be used in the laboratory include the egg parasitoid Trichograma, the pathogens Bacillus thuringensis and nuclear polyhedrosis virus, and the nematode Neoapplectana carpocapsal. The nematode is also the vector for another pathogen, Achromobacter nematophilus. The system is documented in Starks and McMillian 1967.

(3) Alfalfa-aphid-parasitoid. The alfalfa aphid, Therioaphis trifolii (T. maculata) is a sucking pest of leguminous crops. It is exploited by the parasitoids Praon exsoletum (P. palitans), Trioxys complanatus (T. utilis), and Aphelinus asychis (A. semiflavus). This system presents the possibility of testing the effects of chemicals on competition between the parasitoids as well as on the plant-herbivore-parasitoid food chain. These insect species are not available from stock cultures. The system is described in Force and Messenger (1964a, 1964b, and 1965).

(4) Plant-brown scale-exploiters. The brown soft scale (Coccus hesperidum) is a sucking herbivore that can be raised on numerous plant genera including Coleus, Begonia, Ficus, and Ilex. It has more than 35 hymenopterous parasitoids and several coccinellid predators including Chilocorus stigma. Advantages of scale insects as test organisms include:

1. Their sessile nature provides for manipulation and quantification of many population parameters.

2. They share an intimate spatial and chemical relationship with their host plant including a high sensitivity to host chemistry such as concentrations of nitrogen and pesticides.

3. They leave a permanent record of survival and parasitism.

4. They have numerous predators and parasites and engage in intense intraspecific and interspecific competition.

5. They are easily reared in the greenhouse and produce several generations per year.

The major disadvantages of this system include the relatively small amount of work that has been done with the system and the relatively high level of specialized training required in identifying and manipulating the insects.

(5) Housefly-blowfly-parasitoid. These systems include parasitism of the housefly (Musca domestica) by a wasp (Nasonia vitripennis), competition between the housefly and blowfly [Phaenicia (Lucilia) sericata Meig.], and competition in the presence of the parasitoid. These systems require little equipment and have well-defined media; the physical parameters have not been studied in detail as they have for Tribolium but they are probably somewhat less important. Although the population system is fairly simple to run and requires little training, it would require a fair amount of labor. Differences among fly strains used could influence results because flies vary in their sensitivity to parasite attack and can evolve defensive mechanisms. Both fly species are commercially available. Documentation for the fly-parasitoid system is found in Chabora and Pimentel (1970); for the fly-fly system in Pimentel et al. (1965); and for the fly-fly-parasitoid system in Cornell and Pimentel (1978).

(6) Flour beetle competition. Competition between Tribolium castaneum and Tribolium confusum is one of the best studied systems in population ecology. Under certain well-defined conditions, the outcome of the competition is indeterminate. Therefore, the system is thought to be very sensitive. A provisional protocol for this system is presented in Section 7.5. Procedures are available to rid cultures of the pathogen Adelina, but it may be intentionally included as an additional interaction.

### 7.4.2 Promising Systems

Several arthropod interactive systems were enumerated that have good promise, but that are either limited by the unavailability of documentation for standardization or present potential problems for implementation. These systems are discussed briefly in the following sections.

(1) Plant-herbivore-exploiters.

Hemlock scales. This system includes forest ecosystem organisms rather than agricultural pests. Several interactions are possible involving the insect, plant, natural enemy, and competitors. The sessile nature of scales renders them amenable to laboratory testing. The system is documented by McClure (1979a and 1979b).

Gypsy moth. This insect is well documented because of its periodic pest status. It is a forest ecosystem, chewing herbivore. Methods for culturing and manipulation are documented, but there are possible quarantine problems (Campbell and Podgwaite 1971; Capinera and Barbosa 1977; Odell and Rollison 1966; Hugh and Pimentel 1978).

*Grasshopper-grass*. This system provides a laboratory test for plant-chewing herbivore interactions from a grassland ecosystem. The test procedure is described in Dyer and Bokhari (1976).

*Corn rootworm-grass*. This system provides a laboratory test for interactions between a plant and a soil dwelling, herbivorous insect. It is also possible to add an insect pathogen to the system. This system is well documented (Branson 1971; Ortman and Branson 1976).

*Plant-Japanese beetle-pathogen*. This is one of the best documented insect-pathogen systems and provides the advantages of a soil-dwelling insect. Documentation for this system is old but voluminous (Fleming 1963; Hawley 1952; Hadley and Fleming 1952).

*Cactus (Opuntia) - moth or scale-exploiters*. This system includes arid ecosystem organisms and allows several interactions including the use of both chewing and sucking insects on the same plant. Possible herbivores include *Cactoblastis cactorum* (Lepidoptera: Phycitidae), *Dactylopius opuntiae* (Homoptera: Dactylopiidae), *Archlagocheirus funestus* (Coleoptera: Cerambyeidae), and *Metamasius spinolae* (Coleoptera: Curculionidae). Documentation of laboratory procedures may be found in Gunn (1974); Hoffman (1977); Lindley (1978); Moran and Annecke (1978).

(2) *Predator competition*. This interaction is not included in the proposed tests, and we know of no documented systems. One possible system would include checkered beetles (*Cleridae*) and bark gnawing beetles (*Ostomidae*), which are both polyphagous predators living on trees or in the bark. Another possible system would include ladybird beetles (*Coccinellidae*) and green lacewings (*Chrysopidae*), which both feed on aphids.

(3) *Mutualism*. No examples of mutualism are included in the proposed tests. A good example might be the cultivation and consumption of the fungus *Fusarium* by the ambrosia beetle (*Xyleborus ferrugineous*) (Norris and Baker 1967 and 1968; Norris and Chu 1970).

(4) *Plant competition mediated by insects*. This system of a greenbug on small grains includes a class of interactions not included in the other tests. (The plant interactions group pointed out that herbivorous insects can easily be introduced into plant competition experiments by neglecting to fumigate the greenhouse.) It should be easy to extend this test to include other herbivores or natural enemies of the herbivores. Procedures are described in Windle (1979).

(5) *Insect-pathogen*. Dermestid beetles can be cheaply and easily reared. The pathogen *Gregornia* is known to have chronic effects on population parameters such as fecundity and longevity.

Procedures for this system are described in Schwalbe et al. (1973a and b) and Schwalbe et al. (1974).

(6) Interspecific competition. Competition between *Drosophila* species is as well studied as *Tribolium* competition and is also probably a good candidate system. However, we are not sufficiently familiar with the system to evaluate it in detail or to propose its development.

### 7.4.3 Protocol Development (*Tribolium* competition)

(1) Test descriptions. The only system for which a tentative protocol could be developed during the workshop was *Tribolium* competition. The *Tribolium* experimental model has been used extensively to study competition. The system has both competitive and predatory interactions because adult beetles feed on eggs and larvae.

A hypothesis to be tested by this protocol includes - What is the impact of toxic substances on competitive/predatory interactions? Though the system envisioned has only two species, the effects of toxic substances on community diversity, trophic structure, and stability may be inferred from the results.

Size must be considered when designing the system. Under some environmental conditions competitive interactions between *Tribolium confusum* and *T. castaneum* are indeterminate. In other words, the winner cannot be predicted; rather each species wins a given percent of the time. This has been demonstrated in studies by Park (1948, 1954) and Mertz et al. (1976). In standard media (Park et al. 1965), at 29°C, about 25% relative humidity and constant darkness, the two species are eventually matched. In approximately 50% of the cultures *T. confusum* will win, while in the remaining cultures, *T. castaneum* will win.

A description of the recommended test procedure is provided below:

Treatments (a) 10 *T. confusum* and 10 *T. castaneum*
(b) 20 *T. confusum*
(c) 20 *T. castaneum*

Replication. For (a) treatments, a minimum of 20 replicates are needed. For the (b) and (c) treatments, 10 should be sufficient. Replicability can be increased by the use of block design.

Introduction of test chemicals. It would be easy to add the test chemical to the flour medium. Since the medium is usually changed every 30 d when a census is taken, the exposure could be short- or long-term. Exposures of less than 30 d are also possible.

Measurement of effects. Censuses of adults will be taken every 30 d. Immatures can also be speciated but with less accuracy. Counting immatures is also much more time-consuming.

Outcome of competition. In the control (a) treatment, competitive indeterminism should be found, whereas, in the experimental (a) treatments, deviations from the control treatment are a measure of the impact of the test chemical. For example, one species might win in all of the cultures (determinate competition). Time to extinction of one competitor could also be a response criterion for treatment (a).

(2) Analysis of results. There are numerous statistical tests for analyzing results by comparing a control and an experimental treatment (e.g., Dunnett's Test and Duncan's Test) and for comparing several treatments (e.g., Tukey's Test or Scheffe's Test) (see Steele and Torre 1980; Brown 1965). The above test could be used for the (b) and (c) treatments, where each species is alone.

For comparing the (a) treatments, possible goodness of fit test would be appropriate, for example, the G-statistic (Sokal and Rohlf). The control treatment could be used to generate expectations.

If in control (b) and (c) treatments, one species always becomes extinct or if there is a lack of indeterminacy in the control (a) treatment, results should be rejected.

The *Tribolium* experimental model is a "laboratory model system." As such, it is simpler than nature, though it is by no means simple. The results of experiments utilizing the *Tribolium* model may be used to indicate the possible effects of a test chemical on natural communities.

There are several major questions that need to be resolved. For example, how quickly can you predict the outcome of a competition culture? By the third census (90 d), the outcome can be predicted with perhaps 80% accuracy by using numerical superiority as the prediction criterion; by day 150, predictions are perhaps 90% accurate. Exactly how accurate are these estimates?

It might be very useful to use this test system with a test chemical whose "effects" are well known. This would give a better understanding of the ability to generalize results to other terrestrial systems.

Numerous review articles are available, several of which deal specifically with competition: Sokoloff (1972, 1975, and 1978); King and Dawson (1971); Mertz (1972); Park (1948, 1954); Mertz et al. (1976); and Neyman et al. (1956).

## 7.5 Conclusions

The potential test systems for microbial population interactions and community properties are not highly recommended for the following reasons:

1. Population interactions and community properties of terrestrial microbes are difficult to measure because of interference by the soil. These properties can be far more easily measured in aquatic systems if they are deemed to be of interest.
2. The primary importance of terrestrial microbes is their functional role in the ecosystem. Functional responses are also more easily measured (Section 5).
3. Because the relationship between population and community level properties of soil microbes and their functional dynamics in ecosystems are poorly understood, the results of these tests would have little explanatory or predictive power.

The two best potential test systems for microbial population interactions appear to be lichen mutualism and the antagonism between Trichoderma and Rhizoctonia. These systems are well understood and could be easily tested.

Of the plant and microbe systems evaluated by Working Group B, the clover-fescue interference system received the highest rating because it combines interactions between plant populations with interactions between plants and microbial symbionts; it is a well-developed system of some importance and it appears to be sensitive. The agricultural soil microcosm is rated relatively low because it is untried. The remaining systems that include interactions between single plant species and their mutualistic symbionts or pathogens were all thought to hold high promise.

Working Group C (arthropod interactions) concluded that, in general, systems involving Homoptera will be more sensitive than those involving lepidopterous larvae. Systems involving more than one interaction are presumably more realistic but may require much more time, cost, development, etc. There is no clear perception that one or a few particular types of interaction (e.g., plant herbivore; natural enemy-prey) is superior to the others. The major common parameters for all interactions are fecundity, survival, and development time. These should be determined for any test to ensure that effects are detected. However, as systems are developed it may occur that one or more parameters are impractical. For example, measurement of fecundity and development time for nematodes in the earworm-nematode-bacterium system would seriously complicate use of the system.

Some of the serious questions that were left unresolved are:

1. What is the most appropriate method for applying the test chemical?
2. What are the criteria for validating these systems in the field?
3. What magnitude of effect is significant?

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# METHODS FOR MEASURING EFFECTS OF CHEMICALS ON AQUATIC POPULATION INTERACTIONS

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## SECTION 8

## METHODS FOR MEASURING EFFECTS OF CHEMICALS ON AQUATIC POPULATION INTERACTIONS

### 8.1 Introduction

This workshop was convened to discuss laboratory test systems that address interactions among populations of two or more aquatic species. The interactions of greatest concern were predation (especially among fish) and competition (especially among algae), because these phenomena have received the most attention in previous research. Simple laboratory systems containing algae, grazers, and decomposers (referred to hereafter as "multispecies systems" or as "microcosms") were also discussed; there is no sharp distinction between these systems and the mixed flask cultures considered in Section 6. Other population interactions (parasitism, grazing, and symbiosis) have been largely neglected in environmental toxicology and have, therefore, received little attention in this section.

The workshop objectives were: (1) to ensure that the critical review conducted by Oak Ridge National Laboratory (ORNL) of existing and potential methods for testing chemicals for effects on aquatic population interactions was as complete and comprehensive as possible; and (2) to gather information and ideas on the practical applications of such research for hazard assessment under the Toxic Substances Control Act (TSCA) of 1976.

### 8.2 Results and Discussion

#### 8.2.1 Evaluations of Test Methods

Two working groups were formed, one to discuss predation experiments, and the other to discuss competition experiments and multispecies culture systems. Each group evaluated selected test methods in terms of the criteria suggested for hazard assessment protocols. These evaluations (Appendix A) aided in the preparation of the ORNL review (Giddings, 1981). Summaries of the evaluations are presented in Tables 8.1, 8.2, and 8.3. The criteria and rating scales were as follows:

1. Replicability. How similar are the results of replicates from any given experimental run? G = Good, F = Fair; P = Poor; U = Unknown.

2. Reproducibility. How well can an experiment be repeated to give the same results? G = Good; F = Fair; P = Poor; U = Unknown.

TABLE 8.1. EVALUATION OF PREDATION TESTS[a]

| Reference[b] | Replicability | Reproducibility | Standardization | Sensitivity | Time required | Cost | Facilities and skills | Extrapolation |
|---|---|---|---|---|---|---|---|---|
| 2 | G | U | G | U | D | H | Y | G |
| 6,7,8 | G | U | G | H | H | M | Y | P-G |
| 10 | G | U | F | H | W | M | Y | F |
| 11 | G | U | G | H | W | H | Y | G |
| 17 | G | U | G | H | D | L | Y | G |
| 19 | U | U | G | L | D | H | Y | F |
| 20 | U | U | G | H | D | L | Y | G |
| 27 | U | U | U | U | H | L | Y | U |
| 39 | G | U | G | H | D | M | Y | F |
| 40,41 | G | U | G | U | H | L | Y | P |
| 42 | F | U | G | H | D | L | Y | G |
| 49 | G | U | G | U | H | L | Y | U |
| 4,5,50 | G | U | G | U | H | L | Y | U |
| 51 | G | U | G | H | D | L | Y | G |

[a] See text for explanations of criteria and symbols.

[b] See Section 8.4.

## TABLE 8.2. EVALUATION OF COMPETITION TESTS[a]

| Reference[b] | Replicability | Reproducibility | Standardization | Sensitivity | Time required | Cost | Facilities and skills | Extrapolation |
|---|---|---|---|---|---|---|---|---|
| 3 | P | P | P | L | M | H | Y | G |
| 13 | U | U | G | H | W | L | Y | U |
| 14 | F | U | P | U | M | L | Y | P |
| 18 | P | U | P | L | W-M | M | Y | F |
| 23 | G | U | G | U | W | L | Y | U |
| 24 | G | U | G | H | D | L | Y | G |
| 25 | F | U | P | H | D | L | Y | U |
| 26 | F | U | P | U | W | L | Y | U |
| 28 | F | U | G | U | M | L | Y | U |
| 30 | U | U | G | H | D | L | Y | U |
| 31 | U | U | P | U | W | M | Y | F |
| 12,38 | G | U | F | H | W | L | Y | U |
| 46 | U | U | G | U | W | M | Y | G |
| 47 | G | U | G | U | W | L | Y | G |

[a] See text for explanation of criteria and symbols.

[b] See Section 8.4.

## TABLE 8.3. EVALUATION OF MULTISPECIES CULTURE SYSTEMS[a]

| Reference[b] | Replicability | Reproducibility | Standardization | Sensitivity | Time required | Cost | Facilities and skills | Extrapolation |
|---|---|---|---|---|---|---|---|---|
| 21,22 | P | U | P | L | M | M-H | Y | U |
| 32 | U | U | U | U | M | L | Y | U |
| 33 | P-F | U | F | U | M | L | Y | P |
| 35 | U | U | P | U | M | L | Y | U |
| 36,37 | P | U | P | U | M | H | Y | U |
| 43 | G | U | U | U | W-M | L | Y | F |
| 44,45 | G | U | G | H | W-M | L | Y | F |
| 48 | U | U | G | U | D | M | Y | P |

[a] See text for explanation of criteria and symbols.

[b] See Section 8.4.

3. Standardization. How readily can the procedure be standardized for use by other laboratories? G = Good; F = Fair; P = Poor; U = Unknown.

4. Sensitivity. Does the test system show effects at low concentrations of chemical? H = High (more sensitive than acute $LC_{50}$); L = Low (less sensitive than acute $LC_{50}$); U = Unknown

5. Time required. How long does the experiment last? D = 1 to 10 d; W = 1½ to 8 weeks; M = 2 or more months.

6. Cost. What is the cost of setting up one experimental system? Once the system is in place, what is the cost per chemical tested? H = High; M = Moderate; L = Low.

7. Special facilities and skills. Does the procedure require any special equipment, techniques, or training? Y = Yes; N = No.

8. Extrapolation. How well can the results of the laboratory experiments be used to predict chemical effects or other ecological phenomena in natural ecosystems? G = Good; F = Fair; P = Poor; U = Unknown.

In general, predator-prey tests appear to be more replicable and more readily standardized than competition tests or multispecies culture systems (Tables 8.1, 8.2, and 8.3). Reproducibility is virtually unknown for all systems reviewed. Predator-prey tests are more sensitive to chemicals than acute single-species bioassays in many cases; the sensitivity of competition tests and multispecies cultures is largely unknown. Predator-prey tests are the most rapid, usually lasting from less than an hour to several days, whereas competition tests take weeks and most multispecies culture experiments last for months. Few of the test systems in any category are rated highly expensive, but absolute costs per test are generally not known. All tests require some special facilities or skills (see Appendixes for details). Extrapolation to nature is still a matter of guesswork for most systems, although laboratory-field comparisons have been made in a few instances. Overall, predator-prey systems are in a more advanced stage of development as chemical effects tests than are the other two categories. It should be remembered, however, that the different categories of tests were evaluated by different groups of researchers with different backgrounds and biases.

### 8.2.2 Group Discussion

Each working group was asked to (1) identify major issues in multispecies toxicity testing, (2) identify problems in predicting ecological effects of toxic chemicals from results of laboratory tests, and (3) outline research that is needed. The two groups took rather different approaches. The Predation Group concentrated on formulating guidelines for the design and analysis of predator-prey experiments. The Competition/Multispecies System Group addressed some of the more general problems of using complex experimental systems to test chemicals for ecological effects. The following summaries were adapted from the statements of Dr. Charles Coutant, who led the Predation Group, and Dr. Frieda Taub, head of the Competition/Multispecies System Group, that were presented during the final session of the workshop.

(1) Predation experiments. The scope of this session included the relatively simple experiments that have been conducted with one predator and one prey species. The more complex systems that were discussed by the Competition/Multispecies System Group were excluded. Predator-prey tests are seen as a step towards ecological realism. Predator-prey tests are often more sensitive than conventional acute toxicity tests. Finally, predator-prey tests provide data for impact assessment questions related to the survival of prey and the energetics of predator populations.

These tests are not to be performed in a vacuum, but in conjunction with conventional methods. Results must be related to acute or chronic mortality. In a sense, predator-prey experiments are a test of the application factor concept. Predator-prey tests might be an alternative to chronic toxicity tests, because chronic exposure tests are often long and expensive while some predation tests can be completed in a short time.

Where in the testing hierarchy, then, do predator-prey tests fit? Acute $LC_{50}$ tests should be conducted first to provide background information, particularly for predators and prey that might be used in predation experiments. The purposes of the acute tests are: (1) to characterize the relative sensitivity of species; (2) to look for behavioral clues as to what effects might occur in a predator-prey situation; (3) to examine water quality effects (temperature, salinity, turbidity, etc.) that are difficult to include in a predation test but that require investigation; (4) to decide which organisms to use in a predator-prey test; and (5) to determine the necessity for a predator-prey test for a given chemical. Predator-prey tests might be followed by the multispecies tests discussed in Section 8.2.2(2). Results could be used for an impact analysis in terms of the population dynamics of the prey and the bioenergetics of the predator. Ultimately, ecosystem studies will be needed for verification of predicted impacts.

One category of available tests includes single-species tests that are based on the premise that the observed responses might influence predator-prey interactions. Such responses include: swimming speed (burst and stamina), maneuverability, activity, burrowing rate, reaction time, reaction distance, feeding orientation, schooling behavior, aggressive behavior, and learning. At some point in the development of such tests, the effects should be correlated with the presumed predator-prey effects. Another category of tests includes tests with two species, many of which are described in the literature (Appendix A). Usually the prey are stressed separately from the predator, and the predator and prey are then placed together to test the differential selection of the prey by the predator (e.g., Coutant 1973). In a few cases, simultaneous stresses are given to the predator and the prey (e.g., Woltering et al. 1978). We are not aware of any tests in which only predators were stressed. A third category of test involves multiple predators and prey (e.g., Farr 1978).

(a) Design of predator-prey experiments. Criteria that should be used in setting up the optimal system for testing predator-prey relationships include: (1) criteria for the test organisms; (2) criteria for the test systems; and (3) criteria for the test protocols themselves.

The criteria for test organisms are:

1. The organisms must be readily available. Wild strains are desirable, although cultured stocks are more practical for routine testing. The history of cultures should be known.
2. The organisms must survive under laboratory conditions. Wild strains should be given time to acclimate to the laboratory.
3. The predator should be a good feeder under experimental conditions.
4. Short generation times are desirable for organisms cultured in the laboratory.
5. Relatively small animals are more convenient to work with.
6. The organisms should have ecological, economic, or social significance.
7. The organisms should have a realistic potential for exposure to the chemical (e.g., aquatic insects may be unintended targets of arthropod toxicants).
8. There should be a natural relationship between predator and prey. The vulnerability of the prey to the predator should be known.
9. The natural behavior of the organisms should be known.

10. Responses to selected standard chemicals ($LC_{50}$ and chronic toxicity) should be known.

11. The levels of disease and parasitism in the test populations should be as low as possible, unless these factors are to be studied as part of the test (Butler and Millemann 1971).

The criteria for test systems include:

1. The system should be matched to the natural predator-prey interaction. The system must be appropriate to the behavior of the organisms in terms for example, of shelter, substrate, light regime, relative sizes of predator and prey, and ratio of predators to prey.

2. The test should be applicable to a wide range of chemicals.

3. The system should be amenable to a range of concentrations and exposure times.

4. The dosing system should be realistic. Flow-through dosing systems are necessary for long-term tests. The use of carriers for test chemicals should be minimized.

5. Concentrations of the test chemical and its derivatives should be measured periodically during the test. Experience with many chemicals indicates that concentrations are often not constant, especially in static systems.

6. There should be minimum contamination of the untreated portions of the system. For instance, treated prey should not contaminate the water and thereby cause the predator to be exposed to the chemical. This may be difficult to achieve in static systems.

7. The organisms should be behaviorally isolated and free from outside noise and distraction.

8. The system should be replicable.

The criteria for test protocols include:

1. Temperature, salinity, hardness, and other experimental conditions must be controlled. If the toxicity of a chemical is suspected to be greatly dependent on certain physical or chemical variables, it may be desirable to run the predation test under a range of

conditions. Water quality parameters should be measured throughout the test.

2. Manipulations of the test organisms should be minimized. Test organisms must be allowed sufficient time to acclimate to the experimental system. Handling stress should be equalized between predator and prey, if possible. When it is necessary to handle one species more than the other, there should be less handling of the species that has been exposed to the chemical. For example, if one is attempting to quantify effects of the chemical on the predator, the predator should be acclimated to the experimental system before prey are added. This will minimize synergistic effects of handling and the chemical.

3. Internal controls are desirable. The predator should be allowed to feed on stressed and unstressed prey simultaneously. This requires a differential marking technique, which may be difficult with small organisms.

4. Separate prey controls without predators or chemicals should be run simultaneously to correct for normal mortality.

5. Chemical concentrations should range from a no-effect level to one that produces clear effects. Failure to do this has been a deficiency of many papers in the literature (Coutant et al. 1979).

6. Effects of satiation of the predator should be accounted for. The feeding regime must be standardized so that the organisms are neither overfed nor starved. This has to be tailored to the organisms used.

7. Predators and prey should not be reused in successive tests. Learning and accumulation of the test chemical are two ways in which reusing test animals may influence the results (Ginetz and Larkin 1976). Tissue residue analysis should be conducted after long exposure tests where significant accumulation could occur.

8. The endpoint of the test should be more sensitive than an acute $LC_{50}$, and no less sensitive than a chronic test, for most chemicals.

9. Standard statistics should be sufficient for data analysis. Controls should be compared among experiments to determine the inherent variability of the predator-prey response.

(b) Role of predator-prey tests in the hazard evaluation sequence. Predator-prey tests can be sequenced to provide the most information to the impact analyst. The relative sensitivities of the organisms can be determined from $LC_{50}$ tests. The next step would be a predator-prey test, exposing either the prey, the predator, or both to the chemical. The purpose of this test would be to determine if the predator-prey interaction is likely to be affected at levels below the $LC_{50}$.

Effects on survivorship and mortality of the prey can become inputs to population dynamics models. Here, the analyst must consider possible compensatory mechanisms operating on the prey. Some increase in prey vulnerability might be offset by other factors without affecting the dynamics of the population, so that the no-effect level measured in the experiment would be lower than the true no-effect level in the ecosystem. Predator-prey switching could be another compensatory mechanism (Farr 1978).

Changes in the predator's feeding behavior could be incorporated into a predator growth and energetics model. If the predator is affected, there may also be increased survival of prey. A decision must then be made as to which effects are of real concern. Some predator-prey tests might be performed because of interest in the prey; in other tests the predator may be of greater interest.

Ultimately, the validity of predator-prey test systems must be verified in studies on streams, ponds, or other whole ecosystems.

(c) Potential difficulties. One difficulty with any laboratory test is determining whether the laboratory derived no-effect level is really significant in nature. For example, most test systems are designed for one predator and one prey, but in reality predators have a suite of prey available. Once predators eliminate one prey species, they can simply switch to another. This leads to the problem of predicting community dynamics. There are changing levels of prey availability and vulnerability in any natural system, which we are not able to simulate in any simple test.

Another difficulty in predator-prey systems is accumulation of the chemical in the predator. If a predator is perpetually selecting the most contaminated prey, its body burden may continually increase. What will be the effects of the body burden? Clearly, there has been little experience using predator-prey systems to test the effects of organic chemicals.

(2) Competition experiments and multispecies cultures. This group addressed the general problem of predicting ecological effects from laboratory results. Very simple systems, with relatively few organisms, are amenable to mechanistic understanding and are, therefore, powerful research tools. The limitations of simple systems stem from their incomplete connectiveness. The importance of

connectiveness in determining the effects of disturbance on an ecosystem, especially a simple system, is illustrated by a hypothetical example from a recent paper by May et al. (1979). In this example, harvesting of one species results in the extinction of a competing species as a consequence of competitive interactions at a lower trophic level. The situation is unique because the result depends on the hypothesized feeding and competitive interactions of the four species involved. If a natural system were controlled by this kind of connectiveness and one tested a chemical on a laboratory system with a different connectiveness, then it would be impossible to extrapolate from the laboratory to nature. It was suggested that natural systems may have a multitude of possible connective relationships and that natural systems may be more like one another than are simplified systems. This problem, although an important one in the long run, is not easy to deal with.

(a) Complexity. Comparisons of simple and complex laboratory systems with natural systems are major research priorities. If simpler systems are amenable to mechanistic understanding, clear-cut demonstrations of this fact are needed. For example, there are only a few examples in the literature of reversals of competitive dominance due to selective toxicants (Fielding and Russell 1976; Fisher et al. 1974). If these simple systems are sensitive to chemicals, data are needed to prove it.

Ecological complexity in laboratory systems might be achieved in two ways. Synthetic (or gnotobiotic) systems can be made more complex by adding more species. Other systems may be complex by virtue of being naturally-derived. A difficulty with naturally-derived systems, at least with many of those that have been used in the past, is that one cannot always analyze or document the connective relationships within the systems. Each of these approaches (simple and complex) has its advantages as well as its disadvantages. Simpler systems are easier to analyze; complex systems may be more realistic.

(b) Sensitivity. The relative sensitivities of different laboratory systems is another important issue for research in the near-term. (The relative sensitivity of laboratory systems vs. natural environments is the ultimate question, but not one that we are ready to approach yet.) It is important to recognize that sensitivity is partially a function of the experimental conditions; this must be taken into account when comparisons are made among different laboratory systems. Moreover, there are a variety of parameters that can be measured in a multispecies system. Many researchers measure photosynthesis and respiration and examine production/respiration ratios; others enumerate populations; others measure nutrient uptake rates. The sensitivity of the system will be a function of which parameters are selected for measurement.

(c) Representativeness. The results of a multispecies test are, to some extent, specific to the organisms in the system and to the experimental conditions. The problem of selecting test organisms that are representative of natural ecosystems affects the design of multispecies systems just as it affects the design of single-species tests. Likewise, one must choose a temperature, pH, hardness, etc., that are typical of the natural environment of interest. Unless a laboratory system has been tested over a range of experimental conditions, one does not know how situation-specific the results of any particular test may be. The effects of system design and experimental conditions should be studied by each researcher for his or her own system.

(d) Chemical exposure. Another important issue is the possibility of transformation of a chemical in the test system. For example, the state of the chemical may change with Eh, pH, or oxygen. Test chemicals may also be transformed by organisms within the system. This is one instance in which multispecies systems can be more realistic than single-species tests. For example, a chemical that is determined to be quite toxic in a single-species test may be readily degraded by other organisms and produce virtually no effects in a natural ecosystem or a multispecies system.

The exposure of organisms in a multispecies system to a test chemical can be influenced by the presence of other organisms and abiotic components. Sediments may absorb the chemical and reduce the exposure to the biota. Herbivores and predators may be exposed to the chemical through the food chain. Interactions like this between the chemical and the various components of the test system contribute to the greater realism of multispecies systems as compared to single-species tests.

(e) Replicability and reproducibility. According to Dr. Taub, the ability to replicate multispecies systems within an experiment is fairly good. Occasionally several replicates in a group will develop differently from the rest and will be omitted from an experiment. Initially, the systems undergo fluctuations with large amplitudes, occurring synchronously in all replicates. Later in an experiment, the amplitudes decrease but replicates become asynchronous, resulting in high apparent variability among replicates. This is especially true of species enumerations; most chemical factors (e.g., phosphate and pH) tend to be fairly consistent.

When streptomycin was tested in two separate experiments, many of the effects were reproduced, though not necessarily on the same day in the two tests. For example, Anabaena was reduced by the streptomycin; this effect was significant from day 7 to day 28 in one experiment, and from day 11 to day 25 in the other experiment.

(f) Other issues. Two other subjects discussed briefly were interactions among chemicals in ecosystems receiving pollution from several sources and problems associated with carrier solvents necessary for testing certain types of chemicals. The carrier problem may be especially serious in systems containing decomposers; some observed effects may be caused by utilization of the carrier as a carbon source by the microorganisms.

(g) Role of multispecies tests in the hazard assessment sequence. Multispecies tests are considered most useful in the intermediate stages of hazard assessment. They should be preceded by acute toxicity tests with single species. Multispecies tests might be supplements or alternatives to some single-species chronic toxicity tests, because the former are less expensive and easier to perform. If chemicals are expected to be transformed in the environment, a multispecies test should precede extensive testing of the transformation products. If a chemical is not highly toxic in acute screening tests, but produces unexpected mortalities in a microcosm, then the formation of toxic transformation products would be suspected and should be investigated. Certainly, multispecies tests should precede impact analysis. They would, therefore, be used in approximately the same position as predator-prey tests.

## 8.3 Conclusions

Perhaps the major problem to be resolved before predation, competition, and other population interaction tests can be incorporated into a hazard assessment process is extrapolation or generalization of experimental results to predict effects in natural ecosystems. To a certain extent this extrapolation can be facilitated by selecting organisms and experimental conditions that are representative of the natural systems of interest. However, the system-specific interrelationships among populations in nature are too complex to reproduce in their entirety in the laboratory. The degree to which the necessary simplicity of laboratory test systems may distort chemical effects on population interactions is not yet known. There is a need for research to (1) compare the sensitivity of simplified laboratory systems to that of natural ecosystems, (2) relate the ecological complexity of laboratory systems (number of taxa or number of functional groups) to their responses to chemicals, and (3) develop models or other analytical approaches to linking laboratory results to predictions about chemical effects in nature.

A hazard assessment sequence should begin with single-species toxicity screening tests before multispecies tests are undertaken. The screening tests are necessary to identify chemicals that are likely to produce ecological effects, to determine the relative sensitivities of different organisms, and to aid in the interpretation of multispecies test results.

The effects of a chemical in an ecosystem depend in part on whether the chemical is degraded, transformed, sorbed by inorganic or organic substrates, bioaccumulated, etc. An ecologically realistic hazard assessment is possible only in conjunction with a realistic exposure assessment. Fate and effects of chemicals are inextricably linked.

Laboratory systems involving predation, competition, and multiple population interactions are available for development as hazard assessment test protocols. Few of these systems have been used for chemical testing, however, and further experimentation is needed on all types of tests before any particular technique can be selected for immediate use.

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# APPENDIX A

## A.1 Predation Tests

### A.1.1 Butler and Millemann 1971

Effect of parasites on swimming ability of salmonids. Single-species of test with implications for predation.

Replicability: Very good. Standardization: Very good. Sensitivity: Unknown. Time required: One to several days. Cost: $6000 to $10,000 (set up); $200 to $400 per chemical tested. Special facilities: Swimming tube. Extrapolation: Very good if the host-parasite system is natural. Comments: This approach determines the effects of a dual stress, one of which is natural (parasite or disease) and the other of human origin (a chemical) or an animal (vertebrate or invertebrate). The results can show synergism, antagonism, or additive effects between two stresses, e.g., activation of a latent infection by the chemical (Pippy and Hare 1969); increased susceptibility to the chemical by an existing infection (Boyce and Yamada 1977); or protection against the parasite by low concentrations of the chemical (Draggan 1977). In the case of swimming ability tests, the criterion of effect is impairment of swimming ability with susceptibility to predation enhanced.

### A.1.2 Coutant 1973; Coutant et al. 1974, 1979

Effect of temperature stress on susceptibility of fish to unstressed predators.

Replicability: Good. Standardization: Good; has been used for both heat shock and cold shock with some variation. Sensitivity: Better than acute $LC_{50}$. Time required: For chemicals, time will be determined by predator satiation; several weeks to train predators to feed in laboratory; actual test takes minutes or hours. Cost: Mainly for tank set up, which will depend on predator and prey used. Cost per chemical depends on manpower and organism rearing. Special facilities: Holding and exposure tanks for fish. Special skills: Fish care. Extrapolation: Good for cases where only prey are likely to be exposed to chemical; artificial when both predator and prey are likely to be exposed together. Comments: This method has certain disadvantages: (1) low dosages can cause stimulation and thus enhanced survivorship of prey; and (2) some behaviors are protective; stressed fish sink out of sight and escape predation. Either of these phenomena can confuse the interpretation of results.

### A.1.3 Farr 1977

Effects of chemicals on susceptibility of grass shrimp to killifish predation.

Replicability: Good. 18 to 20 replicates per concentration per compound. Standardization: Probably not high because of time. Sensitivity: High because of high arthropod sensitivity to organophosphorus compounds used in test. Time required: High because of number of replicates needed. Cost: Moderately high cost per chemical because of time required. Extrapolation: No shelter was provided for the prey. Comments: The same predators were used throughout the test. With many compounds that accumulate this could cause a problem. By dumping the shrimp into the tank the possible added toxicant stress may produce a synergism resulting in behavior that could not be properly eliminated with the control. The necessary number of replicates to avoid the predator affecting the outcome increases the time and cost factor. Using marked prey (treated and control) in the same tank might improve this. The effects of the toxicant on the fish cannot be determined. Farr makes a good point that this test may have limited significance with stenophagic predators--they will eat the shrimp they need whether they have been affected by dosing or not. His follow-up, two-prey study addressed the implications with opportunistic feeders (see Farr 1978).

### A.1.4 Farr 1978

Effects of chemicals on predator choice between two prey species.

Replicability: Good. Standardization: Easy. Sensitivity: Better than acute $LC_{50}$. Time required: Large amount of time and effort. Cost: High. Special facilities: Enough space for 26 55-gal. tanks. Extrapolation: Demonstrates predator switching to behaviorally impaired prey. Good correlation to natural systems. Comments: Very good test to show predator switching. Demonstrated sublethal effects on most sensitive prey. Too much work involved for a standardized test. Fish-fish predation tests are interesting, but both predator and prey may be affected by toxicants at the same concentration. Freshwater predation tests should be developed with the more sensitive crustaceans as prey.

### A.1.5 Goodyear 1972

Effects of stress on susceptibility of fish to predation; refuge provided for prey.

Replicability: Good. Standardization: Good. Sensitivity: Good. Time required: 7-d acclimation; 10-d test. Cost: Low. Special facilities: Holding and exposure tanks. Special skills: Fish care. Extrapolation: The absolute refuge concept cannot be extrapolated to a natural environment. Once off the shelf, prey were consumed very quickly. Comments: Mosquito fish are easily obtained and reared. Largemouth bass are easily obtained and trained as predators. Test geared specifically to strong shelter-seeking behavior of the prey. The basic concept is sound but this study dealt with only quantitative data (i.e., percent survival); alterations in behavior responsible for the increased prey vulnerability were not dealt with. Quick, inexpensive. Intraspecific interactions between prey organisms were not discussed; for example, does the treatment and/or crowding cause the prey to leave the refuge?

A.1.6 Hatfield and Anderson 1972

Effect of chemicals on susceptibility of fish to predation.

Replicability: Possible. Standardization: Good. Sensitivity: Effects at same level as $LC_{50}$. Time required: 24-h exposure; 24-h clean holding water; 24-h predation test. Cost: Higher than Goodyear's because of size. Extrapolation: (1) Natural light regime used. (2) Natural temperature regime for time of test. (3) The use of the same predator throughout the series of tests raises the question of how "natural" the response of the predator becomes after more than one trial. (4) In the field, the predator may not often be completely prohibited from pursuing the prey. (5) Prey were frequently caught by being forced against the side of the pond. Comments: Same concept as Goodyear's, but the system does not offer any advantages and the cost is higher. Behavioral alterations are not well discussed. The test with the compound Sumithion® did not indicate a more sensitive effect than the 96-h $LC_{50}$. However, it still may be of value in that those fish left at the $LC_{50}$ concentration will themselves be more susceptible to predation than clean fish. Note: Although there is only a weak correlation, a swimming stamina test with the same compound indicated a 35% decrease in critical swimming speed of the brook trout at a similar concentration (0.5 ppm). The comparison of these two types of tests may be of some value.

A.1.7 Kania and O'Hara 1974

Effect of chemicals on susceptibility of fish to predation; refuge for prey.

Replicability: Possible. Standardization: Good. Sensitivity: Increased prey vulnerability at sublethal exposure concentrations. Time required: 24-h treatment to prey; 24-h acclimation; 60-h test. Cost: Low. Extrapolation: see Goodyear 1972.

A.1.8 Li and Li 1979 (and others dealing with zooplankton predation)

Survival of zooplankton prey in the presence of a zooplankton predator.

Replicability: Unknown. Standardization: Should be possible. Sensitivity: Unknown, but if species are differentially sensitive to a toxicant then this system may be sensitive to effects. Time required: Short because interactions are rapid. Cost: Very inexpensive. Special skills: Species identification. Extrapolation: Unclear. Comments: An area that deserves work but for which little background work has been completed. Perhaps a late level screening tool as opposed to a quick first level screen.

### A.1.9 Sullivan and Atchison 1978

Effect of chemicals on susceptibility of fish to predation.

Replicability: Good. Standardization: Good. Sensitivity: Exposure levels investigated down to the "no effect" level. Time required: 48-h exposure (acute) or 21-d exposure (chronic), 48-h acclimation, 168-h test. Cost: Initial set up low, labor high because of behavioral observations made. Comments: Statistics used to analyze the data seem to accurately deal with the probability that the next fish eaten will be a treated fish, given the absolute density at the time. Much more appropriate than the standard Chi-square for this type of data.

### A.1.10 Sylvester 1972, 1973

Effect of stress on susceptibility of fish to predation.

Replicability: Good. Standardization: Good. Time required: Short acclimation, exposure less than 1 min. Cost: Very low. Comments: Predators were preacclimated while prey were not. Predators were starved for 7 d before testing--this is unrealistic. No cover was provided for prey--also unrealistic. Survival time of prey was measured in seconds; this does not reflect behavioral effects of the thermal stress on the prey, but stress from initially being dumped into the tank.

### A.1.11 Tagatz 1976

Effect of chemicals on susceptibility of grass shrimp to predation.

Replicability: Fair. Standardization: Good potential except for possible variability of plant survival. Perhaps artificial plants would be better. Sensitivity: Based on an invertebrate, which probably had a lower $LC_{50}$ than the fish. This compound (Mirex®) often produces delayed mortality necessitating a longer testing time. Time required: Moderate amount of setup time because of necessary replication. Cost: Relatively low setup and cost per chemical.

Extrapolation: Good in terms of availability of toxicant to prey through plants, water, sediment--analyses of all these components were performed. See comments. Comments: The test was designed to observe the effects on the prey and not on the predator. Because no chronic or acute data were included on grass shrimp sensitivity, it is difficult to determine the overall sensitivity compared with a standard test, except that essentially sublethal levels were tested.

### A.1.12 Vinyard and O'Brien 1975

"Tilt box" to measure predator's interest in potential prey item.

Replicability: High. Standardization: High. Sensitivity: Unknown. Time required: Seconds. Cost: Relatively inexpensive. Extrapolation: Unclear. A very simple system that may be useful for early screening.

### A.1.13 Vinyard and O'Brien 1976; Confer et al. 1978; Confer and Blades 1975

Measurement of reactive distance in fish (distance between prey and predator at the point where predator first orients towards prey).

Replicability: Results to date indicate quite good replicability if controlled for prey size and predator size. Standardization: Easy. Sensitivity: Unknown. Time required: Relatively rapid. Cost: Low. Extrapolation: Unclear, although reactive distance controls search volume, which is clearly of significance under low prey densities. Comments: Although this test has not been used in toxicity testing, it has been used with light intensity and turbidity as experimental variables. Atchison is currently beginning to examine the impact of copper on reactive distance of bluegill to mosquito larvae.

### A.1.14 Woltering et al. 1978

Effect of chemicals on fish predator-prey system with both predator and prey exposed.

Replicability: Good. Standardization: Good. Sensitivity: Significant impacts at sublethal levels of ammonia. Time required: 10-d exposure. Extrapolation: See comments. Comments: The feature of simultaneously and continuously exposing both the predator and prey makes sense from an ecological perspective. In this experiment, the bass was actually more sensitive than the *Gambusia*, and a density dependent impact was also assessed. This approach likely makes more sense than exposing only the prey, especially in the case of toxic compounds rather than thermal stress.

## A.2 Competition Tests

### A.2.1 Confer 1972

Competition between phytoplankton and attached algae in continuous flow aquaria.

Replicability: Poor. Reproducibility: Low. Standardization: Very low; standard species not used. Sensitivity: Low. Time required: 5 to 9 months. Cost: Fairly high setup cost; extremely high cost per chemical because of length of experiment, number of parameters measured. Special facilities: Greenhouse or other large area with plumbing. Special skills: Radiotracer technique, taxonomic expertise. Extrapolation: Examines relation of attached algae and open water phosphorus concentration to phytoplankton. Similar to shallow ecosystems with heavy bottom growth. Tracer studies could indicate alteration of energy flow because of toxic stress. Extrapolation depends on similarity to natural system (e.g., surface to volume ratio).

### A.2.2 Fisher et al. 1974

Effect of chemical on algal competition in two-species batch and continuous cultures.

Replicability: Not examined (no replicates run); should be fairly good. Standardization: High. Sensitivity: Good--competition effects in continuous culture observed with PCB at 0.1 ppb (close to ambient in some rivers). Time required: 2-3 weeks. Cost: Low. Special facilities: Constant temperature area; microscope. Special skills: Algal culture. Extrapolation: No data; systems very simplified.

### A.2.3 Frank 1957

Competition between two Daphnia species.

Replicability: Began with 8 to 14 replicates; contamination problem over 18-month experiment. Standardization: Complicated. Time required: 70 d. Cost: Low, comparatively. Extrapolation: Species naturally occur in similar habitats but seemingly do not occur together; extrapolation "only at considerable risk of proving wrong," according to the author. Comments: Evaluating the effects of a toxic substance on a multispecies test system seems to be needlessly complicated by introducing the factor of competition between two closely related (congeneric) species.

### A.2.4 Goulden and Hornig 1980

Competition between two zooplankton species.

Replicability: Low; static system varied with change of media; low food every 2 d, high food every 4 d. Standardization: Low; oscillations between replicates very high with different conclusion drawn as to best competitor over time. Sensitivity: Low? Time required: 1 to 3 months. Cost: Moderate (mainly personnel costs). Special skills: Familiarity with cladoceran life history. Extrapolation: Difficult to assess because variability is high; however, the energy reserves may be important when the food (algae) are diminished either by grazing or toxicants. Comments: Variation between replicates was large--population densities of Daphnia were twice as high in one sample as in a replicate.

A.2.5 Kindig 1979

Algal competition in batch culture.

Replicability: Sufficient to show significant effects with ANOVA. Standardization: Good. Sensitivity: Unknown. Time required: Test runs 7 weeks, but effects observable within 2 weeks. Cost: Very low. Special facilities: Constant temperature room. Special skills: Algal culture. Extrapolation: Unknown (see comments). Comments: (1) Results verified predictions from single species assays to the resistance of *Scenedesmus*. (2) Test indicated competitive ability of *Scenedesmus* relative to other species tested and demonstrated competitive reversal. (3) Results from this experiment predicted *Scenedesmus* increases in complex microcosms with streptomycin treatments correctly. It did not indicate that dominance would occur late in microcosm development (7 weeks). Extrapolation to more complex (i.e., natural) systems is open to speculation. Probably could only be used with extreme caution.

A.2.6 Klotz et al. 1976

Algal competition in batch cultures.

Replicability: Good. Standardization: Good. Sensitivity: Lowest concentration of sewage tested (20%) gave results. Time required: 5 to 7 d. Cost: Low. Special facilities: Side-arm shaker. Extrapolation: Field biomass data verified laboratory findings. Other rivers receiving municipal sewage in the northeast have similar situations with *Chlorella* occurring in the effluent plume, diatoms outside the plume. Comments: (1) It is necessary to shake the cultures so the diatom does not attach to the side of the flask. (2) The diatom must be placed in a blender to disperse clumps for accurate cell counts.

A.2.7 Kricher et al. 1979

Effects of chemicals on productivity and diversity in natural algal communities.

Replicability: Fair. Standardization: Poor (inoculum from natural ecosystem). Sensitivity: Good; 1 ppm significantly reduced carbon fixation and decreased the total number of organisms. Time required: 24 h. Cost: Low. Special facilities: $^{14}C$. Special skills: Algal taxonomy. Extrapolation: Possible.

A.2.8 Lange 1974

Algal competition in batch cultures.

Replicability: Fair. Time required: 15 d or less. Cost: Low. Extrapolation: Possible. Comments: Quantification of blue-green filaments is difficult. The accuracy with which this is done may limit usefulness.

A.2.9 Marshall 1969

Competition among zooplankton species.

Replicability: Fair. Standardization: Good. Sensitivity: Unknown for chemicals. Time required: 100 weeks. Cost: Low. Extrapolation: Possible. Comments: System would need to be tested with a chemical before consideration as a test system.

A.2.10 Mosser et al. 1972.

Effect of chemicals on algal competition in batch cultures.

Replicability: Unknown. Standardization: Should be good. Sensitivity: Better than single-species tests. Time required: 4 d. Cost: Low. Special facilities: Coulter counter. Special skills: Algal culture. Extrapolation: Unknown; simple systems may vary with grazing and sediment.

A.2.11 Muller and Lee 1977

Competition among a ciliate, a foramaniferan, and a nematode (all herbivores).

Replicability: Unknown. Standardization: The organisms' habitat seems too ephemeral to promote standardization. Sensitivity: Demonstrated for nutrient quantity. Time required: 42 d. Cost: Moderate? Special skills: Sophisticated culturing techniques. Extrapolation: Authors emphasize they have limited their approach to nutrient factor and population growth results.

A.2.12 Russell and Fielding 1974; Fielding and Russell 1976

Algal competition in batch cultures.

Replicability: Good. Standardization: Moderate. Sensitivity: More sensitive to copper in dual culture than unialgal; changes in competition detected from 10 to 500 ppb Cu. Time required: 35 d. Cost: Low. Special facilities: Constant temperature room. Extrapolation: Was not done, but suggest it would be possible in case of accidental industrial spillage. Comments: (1) Quantification of growth of filaments (as used here) is more difficult than that of unicellular algae. (2) This method compares the performance of a species against one competitor with the performance of the species against another competitor. By this triangular method you obtain more information than by just studying performance of species alone vs. performance against one competitor.

### A.2.13 Tilman 1977

Effect of nutrient regimes on algal competition in semicontinuous cultures.

Replicability: Unknown. Standardization: Good. Sensitivity: Unknown. Time required: 30-40 d. Cost: Moderate. Special skills: Bacteria-free algal culture. Extrapolation: Apparently good. Data inserted into Monod model accounted for 70% of the variance of two species along a natural silicate/phosphate gradient in Lake Michigan (even though the cells used were cloned from a different lake). Comments: The fact that the clones used in the test, even though not from Lake Michigan, accounted for much of the variability in the abundance of those species is indicative of the extrapolative appeal of this approach. It remains to be demonstrated whether changes in resource utilization with toxicant exposure will extrapolate as well.

### A.2.14 Titman 1976

Effects of nutrient regimes on algal competition in continuous cultures.

Replicability: Appears good. Standardization: High. Sensitivity: Unknown. Time required: 3 weeks. Cost: Minimal. Special facilities: Temperature control area. Special skills: Algal culture. Extrapolation: Resource utilization analysis appears to predict boundaries of coexistence quite well. Intuitively, it seems that imposed stresses should alter the competitive balance between species and become evident with this testing approach. Comments: Establishment of new nutrient gradient boundaries allowing coexistence of species in the presence of a toxicant could be valuable in predicting the outcome in natural systems where nutrient concentrations are known.

## A.3 Algae-Grazer-Decomposer Systems

### A.3.1 Harrass and Taub (FDA Contract #223-76-8348)

Mixed culture; algae, grazers, protozoa, fish.

Replicability: Tested; good. Standardization: Fair; used filtered lake water, enriched with nutrients, inoculated with organisms--other lake waters may be different. Sensitivity: Yes, at low doses; high doses were more similar to control. Time required: 1 to 3 months. Cost: $10 per replicate, not counting cost of maintaining stock cultures. Special skills: $^{14}$C, chlorophyll extraction. Extrapolation: Would yield bioaccumulation and consequences; species diversity shifts; may not predict which species in a natural community would predominate.

A.3.2 Kersting 1975, 1978

Compartmentalized autotroph, grazer, decomposer system.

Replicability: Appears poor. Standardization: Poor because of complexity. Sensitivity: Effects observed appear identical to those achieved with single species assays, or even less sensitive in some trials. Time required: 8 to 9 weeks. Cost: Initial cost high; moderate thereafter. Special skills: Algal culturing. Extrapolation: see Ringelberg 1977, 1978. Comments: This system appears less vulnerable to species invasion than the system detailed by Ringelberg but it is still highly complex.

A.3.3 Kindig (FDA Contract #223-76-8348)

One or two algal species grazed by zooplankton.

Replicability: High. Standardization: Good. Sensitivity: Unknown--established for baseline feeding preference only. Time required: 24 h. Cost: Low. Special facilities: Coulter counter. Special skills: Algal culture. Extrapolation: Low, unless species very similar to these are found in the natural system. Would have to work by "ecological analogy." See comments. Comments: The utility of this experiment in toxicity testing is difficult to assess. The fact that the presence of preferred food species increases feeding on the nonpreferred food species by *Daphnia magna* was a surprise, but this, in itself, was sufficient to allow extrapolation of results. Attempts at extrapolation would require knowledge of the competitive relationship of preferred and nonpreferred algal species.

A.3.4 Neill 1975

Mixed culture of microorganisms, naturally derived.

Replicability: Unknown. Sensitivity: Not tested. Time required: Months. Cost: Low. Extrapolation: Possible. Comments: Good potential for combining ecological effects and fate, except that the fish (which is admitted to the microcosm for brief feeding periods) could remove the toxicant.

A.3.5 Nixon 1969

Mixed culture including algae, bacteria, and grazer (marine).

Replicability: Moderate for bacteria and algae, poor for grazer. Standardization: Moderate. Sensitivity: Not tested. Time required: Steady state after 2 months, experiment ran for 5 months. Cost: Low. Special skills: Bacterial and algal culture. Extrapolation: Low, no grazers survived. Comments: Bacteria and algae stabilized after 50 d but all attempts at maintaining a grazer population failed. This suggests some modification of the system would be desirable before further testing.

A.3.6 Reed 1976

Mixed cultures with algae, bacteria, and grazers.

Replicability: Unknown. Standardization: Poor. Sensitivity: Unknown. Time required: 20 weeks. Cost: Low. Special skills: Taxonomic. Extrapolation: Possible. Comments: Systems were not used to test toxicants.

A.3.7 Ringelberg 1977; Ringelberg and Kersting 1978

Compartmentalized autotroph-grazer-decomposer system.

Replicability: Probably low. Standardization: Potential for invading species high. Expect difficulties in standardizing decomposer chamber flowrates. Sensitivity: Unknown--see comments. Time required: Systems were set up with intent to examine stability; ran 3 years. Time required for a chemical test unknown. Cost: Initial costs high. Cost per chemical depends on length of run required, difficulty of cleaning system from previous run, and reestablishing or reexamining baseline performance. Special skills: Bacterial and algal culture. Extrapolation: See comments. Comments: (1) These systems are unique because they enable separate evaluation of autotrophy/ heterotrophy/ decomposer units. This obviously increases ease of sampling and establishment of chemical fate or manipulation of the system. (2) Increased complexity of the system and its vulnerability to species invasion makes replication and reproducibility quite difficult to achieve, as well as expensive to attempt. (3) Use of toxicants in system is feasible, but reuse of systems afterwards would be questionable. (4) The value of this system is for investigations of nutrient enrichment or nutrient ratio studies on the herbivore-autotroph-decomposer relationship.

A.3.8 Taub 1969

Mixed culture including alga, grazer, bacteria; gnotobiotic.

Replicability: Very good. Standardization: Should be excellent, but not tested. Sensitivity: Not tested. Time required: 1 to 6 months. Cost: Low. Special skills: Sterile technique. Extrapolation: Can extrapolate major trophic level effects to the extent that these organisms are typical of natural organisms; i.e., about the same problems as single species bioassays. Comments: Should be most able to be standardized among laboratories because contaminant organisms are excluded. This test is limited to a few organisms that can be cultured in bacteria free culture or with defined bacterial flora. Could combine chemical fate and ecological effects in one test.

A.3.9 Taub and Crow 1978 and 1980 (in press)

Mixed culture, including algae, grazers, protozoa, and bacteria.

Replicability: Usually good agreement among 4 to 6 replicates. Standardization: High. Sensitivity: Fairly good. Time required: 1 to 3 months. Cost: Less than $10 per replicate, excluding cost of maintaining stock cultures. Special skills: Culture techniques, $^{14}C$, taxonomic. Extrapolation: Can predict trophic level interactions (e.g., effect on primary producers, or change in dominance); probably cannot predict which species would become dominant in a specific natural system. Comments: Especially useful for chemicals that are transformed by some organisms to metabolites that have lesser or greater toxicities, or that become more available via bioaccumulation. Also useful for showing indirect effects such as algal blooms if grazer trophic level is destroyed, or altered grazer relationships if algal community is altered. Most "ecosystem level" variables can be measured (e.g., P/R ratio, chlorophyll a, species abundance, species diversity).

A.3.10 Tsuchiya et al. 1972

Grazing by protozoa on bacteria in continuous culture.

Replicability: Unknown. Standardization: High. Sensitivity: Unknown. Time required: Days. Cost: Several $100 initial costs. Special skills: Sterile technique. Extrapolation: Difficult. Comments: Could be used in conjunction with mathematical models to predict effects.

# APPENDIX B

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16. ABSTRACT

Six workshops, held from November 1979 through March 1980, at Oak Ridge, Tennessee, were designed to identify laboratory methods and data evaluation techniques for predicting the environmental effects of chemical substances. Participants discussed assessment and policy requirements of multispecies toxicology test procedures, mathematical models useful in hazard and risk assessments, and methods for measuring effects of chemicals on terrestrial and aquatic population interactions and ecosystem properties.

Methods were evaluated for their potential for standardization and for use in the ecological hazard and risk assessment processes under the Toxic Substances Control Act. Results from the workshops were used in preparing a critical review of Methods for Ecological Toxicology (EPA 560/11-80-026; ORNL 5708). The workshops were primarily used as a mechanism to collect information about research in progress by bringing together investigators presently working with laboratory test systems and data evaluation techniques.

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